Phenotypic and genetic diversity of Arctic charr (Salvelinus alpinus) in the Lake District, UK

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Abstract

The general aim of this thesis was to assess the phenotypic and genetic diversity of Arctic charr (*Salvelinus alpinus*) populations in the Lake District, UK, in order to understand the key evolutionary mechanisms involved in the development of resource polymorphisms and their genetic divergence.

Arctic charr exhibit high phenotypic variability throughout their range. This study assessed the phenotypic variation between three main lakes, Wastwater, Windermere and Coniston Water, as well as within Coniston Water and Windermere, using principal component analysis of morphological traits. The phenotype varied between these three lakes, and evidence of sympatric polymorphisms has been shown in Coniston Water where two morphs occur. These morphs were similar to those that have been repeatedly described in the literature to be a 'pelagic' zooplanktivorous morph and a 'benthic' morph. The dietary analysis, using both stomach contents analysis and stable isotope analysis, found the dietary segregation between the morphs in Coniston Water to be to a lesser extent than described elsewhere, but did however, suggest a generalist vs. specialist situation. Despite the presence of multiple breeding populations in Windermere, the charr are relatively monomorphic with much of the morphological variation being explained by differences between the sexes. However, their morphology, consistent with a zooplanktivorous diet as was described previously, is not in concordance with their present diet, which had a higher proportion of benthic prey. This indicates that at present the association between diet and morphology is not as close in the Windermere population than has been seen in other lakes.

The assessment of ten microsatellite DNA loci allowed the genetic structure of Arctic charr populations within the Lake District to be resolved. All lakes showed significant differentiation. Relatively weak but significant differentiation was also found between the four putative spawning populations in Windermere. Low levels of gene flow between these populations were also found suggesting that although these populations exhibit philopatry, some individuals stray from their natal grounds

during spawning. For Coniston Water, the microsatellite DNA analysis was less conclusive, with the FCA indicating some divergence between the two morphotypes that was not detected in the F-statistics. Reproductive isolation is therefore incomplete, with high gene flow between the morphs, although sample sizes were unfortunately too small in order to test migration rates.

Mitochondrial DNA analysis indicated that all Lake District populations diverged from an ancestral population common to all lakes. The timing of their divergence is consistent with the retreat of the ice and formation of the lakes after the last glacial maximum.

Declaration

The material contained within this thesis has not previously been submitted for a degree at Durham University or any other university. The research reported here has been conducted by the author unless stated otherwise.

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Chapter 1: General Introduction

Understanding the mechanisms by which new species are generated, the process of speciation, remains a central problem in evolutionary biology. For most of the twentieth century, speciation was thought to require the geographic isolation of populations in order to prevent gene flow. In this process, termed, allopatric speciation (Mayr 1942), the isolation of populations leads to their differentiation by the process of genetic drift. If incompatibilities are sufficient to prevent the production of fertile offspring when the populations come into contact they are said to be separate species by definition of the biological species concept (Mayr 1942). It has now become apparent, by the existence of closely related species living in sympatry (Werner 1976, Day et al. 1994, Skulason et al. 1999), that geographical barriers are not necessarily required for the process of speciation and other mechanisms such as natural selection are more important than previously thought (Schluter 1996, 2000). This, along with evidence of adaptive radiations in novel environments, led to theories of ecological processes driving speciation (Schluter 2001).

Ecological speciation is the process of speciation where by divergent selection acting on traits between populations or subpopulations in contrasting environments leads directly or indirectly to the evolution of reproductive isolation (Peichel et al. 2001). The theory, described by Dobzhansky (1951) and Simpson (1953), amongst others, was widely accepted by the middle of the twentieth century, despite little evidence or ways of distinguishing ecological speciation from other mechanisms that might cause speciation such as genetic drift. Recent evidence of ecological speciation, however, has arisen from studies of island models and newly formed lakes (e.g. Smith 1993, Skulason and Smith 1995).

Theoretically, the first stage of ecological speciation is the development of two or more divergent phenotypes within a population (Schluter 2001). This occurs as populations build adaptations to differing environments by natural selection. In vertebrates, these are frequently associated with the environment and foraging

behaviour and are therefore termed trophic polymorphisms (Skulason and Smith 1995). This can then lead to assortative mating of like morphs, promoting the final stage of speciation, which is the reproductive isolation of these divergent phenotypes (Schluter 2001). This usually occurs via mechanisms of both premating and postmating prezygotic isolation (Coyne and Orr 1999). Ecological speciation can occur in allopatry or in sympatry or the process may begin in allopatry and is completed in sympatry. Here reproductive isolation is an incident of habitation and reproduction in different environments, known as the by-product mechanism (allopatry). After the populations come together in sympatry, the process is completed by reinforcement mechanisms, such as a reduction in hybrid fitness (Figure1.1.).

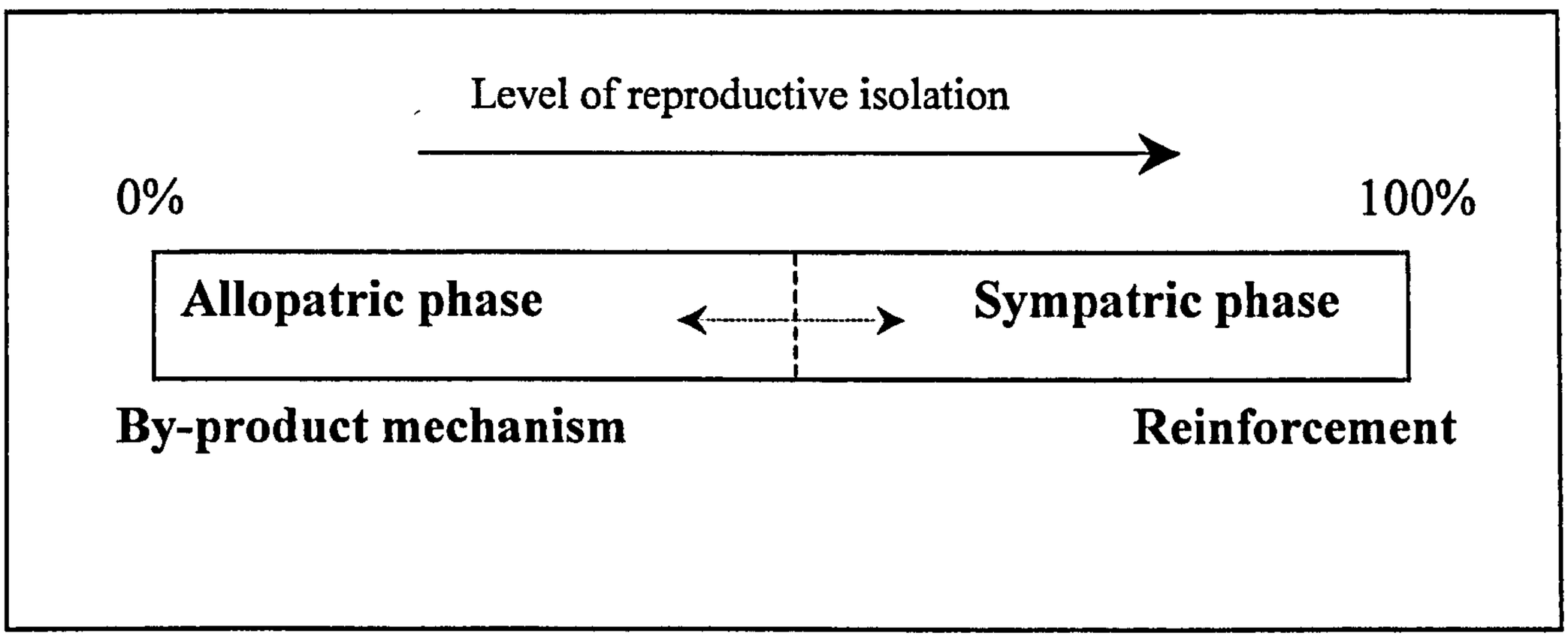


Figure 1.1. The classic scenario of an ecological speciation event, from beginning to end (from Schluter 2001). Reproductive isolation builds in allopatry as a by-product of adaptation to alternative environments. Reinforcement of premating isolation, driven by reduced hybrid fitness, completes the speciation process during the sympatric phase.

The evolution of trophic or resource polymorphisms is relatively common (reviewed in Skúlason and Smith 1995). There are examples from many vertebrate taxa described in the literature including birds (Smith 1993), amphibians (Pfennig 1992), mammals (Skulason and Smith 1995), but most extensively fish (e.g. Schluter and McPhail 1993, Robinson and Wilson 1994). Amongst the examples from fish, well

known and extensively studied systems include the radiation of cichlids (*Cichlidae* species) in the African Rift valley lakes (e.g. Meyer 1987, Hori 1993, Turner et al. 2001, Kocher 2004), and those species inhabiting young postglacial lakes in the northern hemisphere (Schluter 1996).

The African Rift valley lakes formed between 1 and 20 Myrs ago (Turner 1999), although geologists believe that in some cases, for example Lake Victoria, changes in lake level indicate that the lakes that exist now may have formed as recently as 12,400 years ago (Johnson et al. 1996). The cichlid species in the African Rift valley lakes, namely Lakes Malawi, Tanganyika and Victoria, have been a model group for studying speciation and adaptive evolution, due to their huge species richness and morphological diversity (Kocher 2004). The *Cichlidae* represent over 2,200 species, 1,400 of which occur in the African lakes (Turner 2007), but within each lake most species flocks were founded by a single common ancestor (Meyer 1993). The cichlids are known for their adaptive radiations within lakes, largely associated with feeding behaviour. Their ecological and behavioural adaptations to a wide variety of available niches and food resources are coupled with morphological changes to body shape as well as the size and shape of both the jaw and teeth (Turner 2007). Lake Malawi supports the largest radiation and includes scale-eating species, parasite-eating species, piscivores, zooplankton feeders, crustacean feeders, insect feeders, species that sift algae from sediments and those that scrape algae from rocks (Kocher 2004, Turner 2007). The resource polymorphisms exhibited by cichlids have arisen independently in several different lakes but phylogenetic studies show that morphs from different lakes are not closely related, suggesting that they represent examples of convergent evolution (Meyer 1993).

Postglacial lakes in the northern hemisphere formed as the icecap retreated from northern North America and Eurasia about 10,000-18,000 years ago. These lakes were subsequently colonised by freshwater fish from glacial refugia and, where accessible, salt-tolerant species from the sea (Bernatchez and Wilson 1998). Many of these systems are still considered to be in the colonisation phase, where invading species are presented with a diversity of habitats and food resources (Schluter 1996).

This promotes the processes of character release and resource polymorphism (Robinson and Wilson 1995, Smith and Skulason 1996). These lakes provide good systems for studying the ecological processes that drive sympatric speciation, as movement between them is limited, their faunas are generally depauperate, and separate drainages constitute evolutionary independent units (Schluter 1996). The evolution of resource polymorphisms in postglacial lakes has occurred repeatedly and independently in many groups of fish including salmonids (*Salmo, Oncorhynchus, Salvelinus* and *Coregonus* species), the three-spined stickleback (*Gastoresteus aculeatus*), sunfish (*Lepomis* sp.), and smelt (*Osmerus* sp.) (e.g. Bernatchez and Dodson 1990, Day and McPhail 1996, Taylor 1999).

All have common attributes that implicate ecological causes including rapid evolution of species isolation, persistence in the face of gene flow, a high degree of niche differentiation, and high intrinsic viability and fertility of hybrids (Schluter 2001). Ecological theory states that divergent natural selection is the ultimate cause of the development of resource polymorphisms (Schluter 2000). Divergent selection is natural selection that pulls the means of two or more populations towards different adaptive peaks generated by uneven fitness gains at different points along an environmental gradient (Frankham et al. 2002). There are two proposed sources of divergent selection; differences between populations in external environments and both the presence of intraspecific and absence of interspecific resource competition ('ecological opportunity') (Pfennig et al. 2007). Random genetic drift can also contribute to phenotypic divergence by facilitating a population's movement across a fitness valley into the domain of an alternative peak (Wright 1982). The extent to which genetic drift can contribute to adaptive peak shifts is unknown but is likely to be greater when a population is small or when the fitness valley is shallow (Coyne and Orr 1999).

The rapid evolution of reproductive isolation has been indicated by many examples where two very closely related sub-species coexist in lakes less than 15,000 years old. Modern molecular techniques using neutral genetic markers that are unaffected by selective pressures allow the fine scale detection of changes in allele frequencies

within and between populations that indicate genetic differentiation caused by random genetic drift and reductions in gene flow (Frankham et al. 2002). It is therefore possible to detect reproductive isolation between closely related sympatric morphs. For example, three-spine stickleback limnetic-benthic species pairs from Enos and Paxton lakes, British Columbia, are separated by small but significant genetic distances (McPhail 1992). Hendry et al. (2000) tested for intrinsic barriers to geneflow between two populations of sockeye salmon (*Oncorhynchus nerka*) that were introduced into Lake Washington, Washington, US in the 1940s, from a common source. These populations diverged into two populations based on spawning habitat and the significant genetic differentiation found between them, suggested that their reproductive isolation had occurred rapidly, within a maximum of 13 generations.

There are several models of speciation whereby morphotypes can become genetically differentiated (Figure 1.1.), including selection in sympatry, drift in allopatry or a combination of the two. Phylogenetic studies using mtDNA provide evidence of both allopatric and sympatric origins of species pairs of postglacial fishes. For example, a phylogeny based on mtDNA restriction fragments suggested that three-spine stickleback species pairs from the British Columbian lakes, described above, originated independently in sympatry (Taylor and McPhail 1999). However, another study used allozyme frequencies to show that the limnetic species is more similar to the modern marine species, a pattern consistent with a double invasion hypothesis (McPhail 1992). Whether gene flow has diminished gradually between species pairs from initially high levels in sympatry or was moderate to begin with, as in the case of a double invasion, it is clear from the genetic evidence that most species pairs have persisted despite the potential for high gene flow (Schluter 1996). The fact that genetic distances between species pairs or polymorphisms in most cases of postglacial fish are small (< 0.5 % mtDNA sequence divergence; Bernatchez and Dodson 1990; Taylor and McPhail 1999) suggests that even if they were formed in allopatry, this phase was short-lived. Phylogenetic studies of African cichlids have also allowed the determination of their origin and the processes important in their speciation. Many groups of cichlids for example, haplochromines, are monophyletic

(Meyer 1993), suggesting that all the species within a lake are founded from the same lineage. This would suggest that speciation occurred within the lakes after their formation. Allopatric speciation involves geographical barriers to gene flow, which are rare in open water systems. However, in very large lakes with discrete habitats, barriers to dispersal can be formed, isolating populations from one another. A good example of this is the rocky shore cichlids in Lake Malawi, where populations isolated by stretches of sandy sediments show significant genetic differentiation (Van Oppen et al. 1997). They are also rarely observed over sandy sediments suggesting they are significant barriers to dispersal (Turner 1999). In the three major African lakes, there was also the opportunity for allopatric speciation in peripheral lagoons during the major reductions in lake levels that have occurred since their formation. This is termed extralacustrine speciation (Turner 2007), and the most extreme example is of Lake Tanganyika, which was divided into several smaller isolated basins (Sturmbauer and Meyer 1992). There is also evidence for sympatric speciation of cichlids in a number of African lakes. For example, Lake Barombi Mbo is a small crater lake in Camaroon, supporting 11 cichlid species that mtDNA studies indicate radiated from a monophyletic invasion. The lake also has a continuous homogenous shoreline ruling out the possibility of geographical barriers to dispersal, strongly suggesting a sympatric origin (Schliewen et al. 1994).

Resource polymorphisms in fishes in postglacial lakes tend to follow the same patterns of phenotypic divergence along similar ecological gradients and clearly correlate with the number and availability of habitats and food resources in the lake system (Schluter and McPhail 1993). These similarities in different groups of fish suggest that common processes of evolution have been operating. In the majority of lakes discrete limnetic, littoral and deep benthic habitats are found and therefore, morphotypes associated with these habitats coexist. The limnetic morph usually specialises on zooplankton prey, whereas the deep benthic or littoral morphs usually consume larger invertebrates associated with deep sediments or littoral vegetation. For example, where pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*) sunfishes coexist they inhabitat distinct ecological niches (Neff 2004). Pumpkinseeds feed on snails and inhabit the shallow littoral zone whereas bluegills

occupy open-water habitat and feed on zooplankton. In lakes where pumpkinseeds occur and bluegills are absent, pumkinseeds differentiate into two morphs, the typical littoral morph and an open-water morph that occupies the niche usually occupied by the bluegill (Robinson and Wilson 1994, Robinson et al 2000). A consistent set of morphological differences is associated with this habitat and resource split (Skúlason and Smith 1995). Planktivores tend to have slender, streamlined bodies, and narrow terminal mouths. Benthivores generally have a bulkier body shape with large robust jaws suitable for the consumption of invertebrates with hard exoskeletons (Webb 1984, Jonsson and Jonsson 2001). Pumpkinseed sunfish open-water morphs generally have a more slender body form, larger eyes and caudal locomotion that optimises foraging on zooplankton, whereas littoral morphs have deeper body forms, shorter heads, smaller eyes and larger mouths (Gillespie and Fox 2003, Parsons and Robinson 2006). In some species, for example, sunfish (*Lepomis* species) and whitefish (*Coregonus* species), planktivores have more numerous, long and slender gill rakers to sieve and ingest planktonic prey (Langeland and Nost 1995, Gillespie and Fox 2003, Amundsen et al. 2004).

The large degree of differentiation in morphology of the mouth parts of cichlid fish, radiated in response to the utilisation of different niches (Kaufman et al. 1997, Danley and Kocher 2001, Kocher 2004) has also occurred independently in several lakes. These replicated patterns of environmentally related phenotypic divergence in independent species pairs and the fact that the divergence is in traits with strong functional significance is strong evidence of selection. There is a consistent association between particular phenotypes and environments, which is unlikely to have resulted from non-ecological mechanisms that can also cause divergence such as drift or founder effects (Schluter 2000).

Reciprocal transplant experiments, measuring the performance of phenotypes transplanted to an opposing environment, can provide further evidence of divergent selection acting on a population (Malmquist 1992, Day and McPhail 1996). It is expected that transplanting a phenotype to a different habitat where it would have reduced fitness would result in trade-offs. Habitat transplants in pairs of three-spine

sticklebacks revealed steep trade-off in growth rates (Schluter 1995), and reduced foraging success (Schluter and McPhail 1993). The volume of prey ingested per strike in littoral sediments was five times higher in benthic morphs than in limnetic morphs. Conversely the rate of prey capture in open water was three times higher in limnetic morphs than in benthic morphs due to their increased ability to seize small planktonic prey. Studies of other fish species have resulted in similar findings (Robinson and Wilson 1996, Klemetsen et al. 2002). However, studies of pumpkinseed morphs show that trade-offs are not equal in each morph (DeWitt et al. 2000) and usually open-water morphs can feed on both zooplankton and littoral invertebrates but not vice versa. This suggests that feeding on zooplankton is more reliant on morphology than feeding on littoral invertebrates.

The examples above refer only to differences in foraging habitats; differences in breeding habitats also exist but are not as common (Næsje et al. 2004). For example, limnetic and benthic three spine stickleback morphs both breed in the littoral zone but in different microhabitats (Hatfield 1997). There are also incidences of anadromous and lake resident salmon that spawn on different substrates within the same rivers (Hendry and Quinn 1997). Species pairs can also differ in time as well as location of spawning (Skulason et al. 1989, Klemetsen et al. 1997, Adams et al. 1998). For example, the spawning times of three Arctic charr morphs in Loch Rannoch, Scotland, overlap slightly but peak separately between October and November (Adams et al. 1998). The spawning locations are also 12 km apart. This may well have evolved independently to differences in foraging habitats, but the selective mechanisms in this case are unknown. Divergence in foraging niche may have indirectly led to divergence in breeding habitat and time by a number of mechanisms, including, genetic hitchhiking, where genes controlling reproduction are linked to those controlling traits involved in resource use (Rice and Hostert 1993), differing selective pressures on life history, or reinforcement of premating isolation (Schluter 1996).

Although genetic studies have indicated reproductive isolation between sympatric morphs in postglacial fishes, low levels of hybridisation occur, for example, in

natural populations of three-spine stickleback species pairs, about 1-2 % of adults are hybrids, identifiable by their intermediate morphology (McPhail 1992). Laboratory studies have shown that hybrids are fully viable and fertile; F_1 and F_2 hybrids between limnetic and benthic three-spine sticklebacks have a growth rate that is the average of that of their parents and the growth rate of backcrosses is only slightly lower (Hatfield 1997, Hatfield and Schluter 1999). This suggests that extrinsic rather than intrinsic, postzygotic reproductive isolation are among the main mechanisms in force.

Theoretically, the depletion of shared resources will drive species or populations to exploit novel resources where they come under contrasting selection pressures (Forseth et al. 2003, Bolnick 2004), however, implicating competition as the cause of divergent selection is very difficult. Observational evidence of this in natural populations includes exaggerated divergence in sympatry, where phenotypic differences between populations are greater when they coexist in sympatry than when they occur in allopatry. Two European whitefish (*Coregonus lavaretus*) morphs, differentiated in gill raker numbers, have been identified in lakes in northern Norway. In sympatry, the two morphs exhibit strict niche segregation, the densely rakered morph feeding on zooplankton in the pelagic zone whereas the sparsely rakered morph feeds mainly on benthic invertebrates in the littoral zone. In allopatry, however, the densely rakered morph exhibits a larger niche width, utilising both the pelagic and littoral zones and foraging on both zooplankton and benthic invertebrates (Amundsen et al. 2004). This pattern can also be seen in Atlantic whitefish in northern US (Lu and Bernatchez 1999).

Similar patterns are observed in the three-spine stickleback (Schluter and McPhail 1992) where benthic and limnetic populations occur in some lakes whereas solitary species occurring in other lakes tend to be morphologically and ecologically intermediate. It is thought that the solitary form represents the ancestral freshwater sub-species, which was displaced towards a benthic form when the lake was subsequently colonised by the ancestral zooplanktivorous marine sub-species (McPhail 1992). Pritchard and Schluter (2001) carried out experimental studies to

show that the competition experienced by the ancestral marine sub-species was greater when paired with the intermediate species (pre character displacement) than with the benthic species (post character displacement), thus providing further evidence of the role of resource competition in character displacement in the three spine stickleback. A further study aimed to recreate the expected pre character displacement conditions thought to have prevailed when the lakes were first colonised by their marine ancestors (Schluter 1994). Here, addition of zooplanktivorous morphs to habitats containing morphologically intermediate morphs caused differential changes in growth rates of differing phenotypes within the morphologically intermediate population, with selection favouring the benthic phenotype.

Despite the similarities in processes of resource polymorphisms, there are differences among species and localities that reflect varying degrees of differentiation. This may reflect historic differences, for example, the age of the system or different colonisation histories and/or different generation time within and among species (Gislason et al 1999). For example, sympatric forms of the three-spine stickleback are generally considered separate species (McPhail 1992). In Arctic charr (*Salvelinus alpinus*), however, genetic differentiation between sympatric forms varies considerably across systems (reviewed in Chapter Four).

Variation in phenotype may have a genetic basis or alternatively, may result from phenotypic plasticity. Phenotypic plasticity is where a single genotype can produce multiple phenotypes as a function of the environment; it not only involves morphology but also physiological state and/or behaviour (West-Eberhard 1989). Phenotypic plasticity is often adaptive as it allows populations to respond to changing environmental conditions. Thus species living in dynamic environments are likely to evolve increased plasticity (Pfennig 1992). It is not surprising, therefore that fish inhabiting postglacial lakes, having evolved in dynamic glacial environments, have evolved high levels of adaptive phenotypic plasticity (Skúlason et al. 1999). Plasticity in behaviour is often the first aspect of the phenotype to change and initiate new directions of evolutionary change. For example feeding

behaviour during development can extensively influence morphology. Mittelbach et al. (1999) showed that a relatively higher availability of one prey type during larval development induces morphology in the pumpkinseed sunfish adapted to the acquisition of that prey type. It is therefore possible to induce changes in morphology by rearing on certain prey types (Hegrenes 2001, Parsons and Robinson 2007). Heterochrony, the timing of developmental events, is also important in the evolution of morphs (Skulason et al. 1999; Eriksson et al. 1999). Benthic morphs are often paedomorphs that retain juvenile characteristics in the adult morphotype. Heterochrony could be based on a simple change in a regulatory gene mechanism (Snorrason et al 1994; Skulason and Smith 1995), potentially modified by both genes and the environment (Meyer 1987, West-Eberhard 1989).

Common garden experiments, where different morphs are reared in a common environment, and breeding experiments also indicate a direct genetic basis for some phenotypic attributes. Hori (1993) identified a genetic basis for the direction of mouth opening in "left and right handed" scale-eating cichlid fish of Lake Tanganyika. Much experimental work in this area has been carried out on Arctic charr and this is reviewed in Chapter four. Recent genetic mapping studies have provided further insight into the genetic basis of phenotypic divergence. For the three-spine stickleback species, this was made possible by the development of a microsatellite linkage map (Peichel et al. 2001). The genetic basis of several phenotypic traits, including lateral plate number and pelvic reduction (Cresko et al. 2004), in the three spine stickleback have now been identified and their diversity has been attributed to changes at the same loci (Colosimo et al. 2004), highlighting the potential for adaptive divergence from relatively few genetic changes (Bell et al. 2004).

The relative importance of phenotypic plasticity and genetic contribution to the development of resource polymorphisms varies greatly between species and both within and among systems (West-Eberhard 1989), and is dependent on past and present selective environments as well as developmental constraints, such as the cost of plasticity. It is likely that phenotypic plasticity is more important in early stages

of segregation, especially in newly formed lakes, and as the environment becomes more stable and genes are assimilated, plasticity is reduced and phenotype develops a genetic basis.

The Arctic charr (*Salvelinus alpinus* L.) is a stenothermic species from the salmonidae family, inhabiting lakes and rivers throughout the artic, subarctic, boreal and temperate climate regions of the holarctic. It has the most northerly circumpolar distribution of any freshwater fish and has been recorded as far north as Ellesmere Island, Canada (82°34'N) (Johnson 1980). In Europe the southernmost populations are found in the Pyrenees, although most have been introduced by man, and on the American continent populations occur as far south as Maine, US (Kircheis 1989). Within its northern range, above latitudes of 65°N, Arctic charr exhibit anadromous behaviour where the marine environment is accessible. Below this latitude populations are exclusively freshwater. There are estimated to be 50,000 populations worldwide (Maitland 1995) although this is likely to be an underestimate. The majority of populations are found in lakes in Scandinavia, then Canada, Russia, Iceland and Greenland, with fewer populations in North-eastern Central America, Great Britain and Alpine regions. Although exclusively riverine populations do occur, Arctic charr are typically found in cold oligotrophic lakes with depauperate fish communities and in alpine and northern lakes it is often the only fish species. Its native distribution closely follows the deglaciation of the ice sheet (Jonnson 1980) with populations occurring over most of the glaciated areas in early post-glacial times. The species is now absent from the most southern areas of this region probably due to the warmer climate, eutrophication and negative interactions with complex fish communities (Maitland 1995). It is an important species for sport fishing and several commercial fisheries have been established in Canada (Klemetsen et al. 2003).

Consistent with postglacial fishes described above, Arctic charr exhibit great phenotypic and genetic variation throughout their distribution. Extensive literature describing sympatric morphs from northern latitudes is reviewed in Chapters Three and Four. Arctic charr populations in England, confined to eight lakes in Cumbria,

are amongst the most southern of their range at low altitudes. These populations have been little studied, compared to some northern populations, and may provide further insight into the ecological processes involved in the development of resource polymorphisms especially at low latitudes where different environmental conditions, such as lake productivity and species richness, prevail.

Thesis Aims

Broadly this thesis aims to assess the phenotypic and genetic diversity of Arctic charr (*Salvelinus alpinus*) populations in the Lake District, UK, in order to understand the key evolutionary mechanisms involved in the development of resource polymorphism and their genetic divergence.

The effects of global climate change, recently of great concern, may well effect these populations more extensively than those in more northern latitudes and it is as yet unknown what effects factors such as increased surface temperatures, depleted oxygen levels, and changes in species community composition (Winfield et al. 2007), may have on the phenotypic and genetic diversity of such a species. Chapter Two examines the diet of Arctic charr in Windermere and compares it to past information, in the light of recent environmental changes and more specifically will address the hypothesis that increased utilisation of the pelagic zone by roach, changes in lake temperature and oxygen levels will cause changes in the foraging habits of Arctic charr. Chapter Three compares the phenotypic diversity of Arctic charr both within and between the two largest English lakes, Windermere and Coniston Water, in relation to foraging behaviour and diet, in order to assess the relative importance and function of various ecological mechanisms that promote diversification within populations of freshwater fishes. It is hypothesised that a correlation between charr morphology, diet and spawning behaviour will exist in both lakes. Comparisons of the genetic diversity within Windermere and Coniston Water in relation to morphology, provide insight into the evolutionary mechanisms important in the reproductive isolation and speciation of Arctic charr and other freshwater fishes. Chapter Four addresses the hypothesis that any sympatric trophic polymorphisms present within these lakes will be genetically divergent despite the

absence of physical barriers to gene flow, due to reproductive isolation on the basis of differential habitat use or spawning behaviour.

Due to the great genetic and phenotypic diversity of Arctic charr populations there has been much debate over its status as a single species (Maitland 1995). It is very difficult to assign populations for conservation, as each population is to some degree genetically unique. Therefore reductions in population numbers that has previously occurred in some of the English lakes may cause substantial loss in genetic diversity. This, along with the long-term charr fisheries in many of the English lakes increases the importance of managing charr stocks (Winfield et al. 2007). An in-depth study of the genetic diversity of English Arctic charr populations with an aim to identify stocks for conservation is required. Therefore, Chapter Five investigates the origin of Arctic charr in the Lake District and provides information on the genetic diversity between the lakes. This will also provide insight into the evolutionary processes important in the speciation of freshwater fishes and the speed at which this occurs.

Chapter 2 : Long-term changes in feeding ecology of Arctic charr (*Salvelinus alpinus*) in Windermere in response to environmental changes

2.1. Introduction

2.1.1. The population dynamics of Arctic charr in Windermere

The population dynamics and ecology of Arctic charr in Windermere have been studied extensively since the 1930s (for example, Frost 1965, Mills 1989). This long term monitoring indicates there is little impact from the small, semi-commercial plumb line fishery (minimum exploitable length c. 200 mm) (Winfield et al. 2007), which now persists since cessation of the commercial netting fishery in 1921 (Kipling 1972). The population did, however, suffer during the 1980s when regular water quality testing indicated nutrient enrichment of both basins (Mills et al. 1990). This enrichment was largely due to increased use of nitrogen fertilisers within the basin and the input of phosphorus in sewage outflows (Elliott and Baroudy 1995).

Although nutrient enrichment in deep lakes is not directly harmful to charr, the associated hypoxia of bottom waters may impact charr. Salmonids including Arctic charr are known to be sensitive to dissolved oxygen conditions (Alabaster and Lloyd 1980). Laboratory experiments on young charr from Windermere (Baroudy and Elliott 1994) revealed that incipient lethal oxygen levels, measured as survival over seven days, were as low as 1.8 to 2.0 mg L^{-1} at temperatures of 10 °C or below and 2.2 to 2.4 mg L^{-1} at temperatures above 15 °C. These values are lower than have been recorded for other salmonids but do not address the issue of tolerance to low levels of oxygen for prolonged periods. It has been suggested that chronic reduction of oxygen levels below normoxic conditions will restrict the volume of water available to charr, especially in summer when the lake is stratified and they reside in deeper, cooler water (Elliott and Baroudy 1994).

During this period of high nutrient enrichment and low oxygen levels, hydroacoustic surveys carried out in 1988 (Mills et al. 1990) observed that Arctic charr were

displaced from the deepest areas of the south basin. The concern for the Arctic charr populations led to the introduction of tertiary chemical stripping of phosphate at the lake's two sewage treatment plants located in each basin in April 1992. This reduced concentrations of soluble reactive phosphate in the south basin to that of the early 1970s (Parker and Maberly 2000) and appeared to produce a swift recovery of the charr populations in both basins. Jones et al. (2008) used oxygen profiles and monthly hydroacoustic surveys in the years 2002, 2003 and 2004, to investigate the effect of oxygen concentrations on the distribution of Arctic charr in Windermere. Their findings showed that during this period, the lower 90 % bound of Arctic charr vertical distribution was observed at a minimum oxygen concentration of 3 mg L^{-1} in the south basin, suggesting that charr will avoid spending prolonged periods in water below this concentration.

In recent years further environmental changes have occurred in Windermere including an increase in lake surface temperature, an extended period of thermal stratification and the expansion of a non-native roach (*Rutilus rutilus*) population. The overall mean lake surface temperature for the period between 1961 and 1990 was 10.4 °C but for the period of 1990 to 2005 the overall mean increased to 11.5 °C (Winfield 2007). It is unknown what effect this increase in surface water temperature will have on the charr population but the rise in temperature has been sufficient to impact the spawning time of perch (*Perca fluviatilis*) (Winfield et al. 2004a). It is likely that warmer temperatures will similarly affect charr spawning times and also egg incubation periods and the development of charr fry (Frost 1965). Arctic charr may also avoid warmer surface waters possibly due to the higher oxygen requirements at higher temperatures (Elliott and Baroudy 1995) or that growth rate rapidly slows at temperatures above 19 °C (Larsson et al. 2005). Although laboratory experiments have shown that the maximum growth of Arctic charr in Windermere occurs between 14.4 and 17.2 °C (Larsson et al. 2005) actual preferred temperatures can be much lower. Larsson et al. (2005) recorded preferred temperatures for Arctic charr from two populations in Sweden of 10.8 and 11.8 °C. A study of the year round distribution of Arctic charr in Windermere using monthly

hydroacoustic surveys from 2002-2004 (Jones et al. 2008) reported that Arctic charr in Windermere tend to reside in water below the 16 °C isotherm.

Roach was introduced to Windermere in the early 1900s but until the 1990s remained localised and rare (Winfield and Durie 2004). Extensive gill netting in the south basin in 1979 and 1980 failed to record a single specimen (Craig and Fletcher 1981). During the early 1990s recreational angling catches of roach began to increase, especially in the south basin and the increases in total fish abundance recorded using hydroacoustic surveys from 1990 to 2005 have been attributed to increased numbers of roach in offshore surface waters (Winfield et al. 2007). It is thought that the recent increase in the roach population is a result of increased water temperature rather than nutrient enrichment (Winfield et al. 2007).

Winfield et al. (2007) carried out a long-term study of the dynamics of Arctic charr populations in Windermere in response to the impacts of eutrophication, increased water temperatures and the expansion of the roach population. Continuous angler (plumb-line) catch per unit effort (CPUE) data over the period 1966 to 2005 showed little change in charr numbers in the north basin but in the south basin numbers showed some decline in the late 1980s and early 1990s followed by a dramatic decline since 2000. This decline was correlated with a deterioration of oxygen concentrations in the hypolimnion of the south basin to levels similar to those observed prior to the introduction of phosphate stripping (Mills et al. 1990), reducing its suitability as an Arctic charr habitat. Winfield et al. (2007) also hypothesised that competition by zooplanktivorous roach invading the charr's preferred habitat may be a cause of their decline in the south basin.

2.1.2. Feeding ecology of Arctic charr

Arctic charr have been described as generalistic feeders, consuming a wide range of prey including zooplankton, benthic macroinvertebrates, terrestrial insects and fish (Johnson 1980), the importance of which often varies seasonally (Amundsen 1995). Where trophic morphs exist in sympatry, the different morphs will often specialise on separate prey sources, most commonly zooplankton or benthic macroinvertebrates

in order to utilise available niches (e.g. Adams et al 1998). In Windermere, however, no distinct trophic morphs have been found and the whole population has been described as zooplanktivorous for much of the year with a switch to benthic invertebrates such as chironomid larvae for the months from December to April when zooplankton is scarce (Frost 1977). A later study, however, found some differentiation in diet related to the habitat in which the fish were caught (Mills 1989). Here, stomachs of angled fish were found to contain almost exclusively zooplankton whereas the stomachs of charr caught in benthic gill nets contained benthic invertebrates, principally chironomid larvae, with only a minority containing zooplankton.

2.1.2.1. The use of stable isotopes in dietary studies

Examination of stomach contents is a common method for the assessment of fish diet (Hyslop 1980). However, a major disadvantage of this method is that it only indicates very recent diet, which is often not sufficient in species where diet varies widely or consumption is sporadic. This problem is compounded where large sample sizes of fish for dietary analysis cannot be obtained, particularly for species of conservation concern. The application of stable isotope techniques in dietary studies has become increasingly popular over recent decades (Grey 2006). Analysis of carbon and nitrogen stable isotopes is a powerful tool in examining spatial scales of nutrient flow in freshwater food webs, especially when used in conjunction with stomach content analysis (Peterson and Fry 1987).

Phytoplankton and benthic algae have very different $\delta^{13}C$ signatures. Benthic algae generally exhibit less $\delta^{13}C$ fractionation during carbon fixation than do phytoplankton. This is due to the high diffusion resistance of carbon dioxide in water, causing plants with well defined boundary layers to assimilate the otherwise normally depleted $\delta^{13}C$ (France 1995). This is the case for benthic algae, where decreased water turbulence and consequently higher diffusive boundary-layer resistance cause $\delta^{13}C$ enrichment. Benthic algae are therefore usually enriched in $\delta^{13}C$ by ~7‰ compared to planktonic algae. Organisms of the profundal zone of a lake for example, chironomids, generally exhibit highly negative $\delta^{13}C$ ratios possibly

due to the fixation of respired carbon dioxide (Vander Zanden and Rasmussen 1999). $\delta^{13}C$ of consumers is similar to that of their prey, and is therefore, conserved up a food chain (DeNiro and Epstein 1978), allowing the $\delta^{13}C$ value of aquatic consumers to provide information on the sources of energy available to them. France et al. (1995) summarised stable carbon isotope signatures for primary producers and consumers in freshwater aquatic systems (Table 2.1).

Table 2.1. Carbon stable isotope values of freshwater aquatic primary producers and consumers (France et al 1995)

Sample	$\delta^{13}C$ values (‰)
Phytoplankton	-32±2
Epilithic algae e.g. diatoms	-17±2
Epiphytic filamentous algae	-27±3
Littoral consumers	-25 to –26
Pelagic consumers	-33 to –34

Nitrogen stable isotopes are useful in dietary analysis as consumers become enriched in ^{15}N relative to their food by approximately 3-4‰, therefore, the $\delta^{15}N$ signature can be used to estimate the trophic level of an aquatic consumer (Minagawa and Wada 1984). There is however greater within-system variation in $\delta^{15}N$ values for organisms at the base of a food web and therefore a measure of $\delta^{15}N$ must be related to baseline nitrogen values (Vander Zanden and Rasmussen 1999). However, there are many examples of studies where this has not been done (Adams et al 2003, Adams et al 2006) and it is possible to assess variation in $\delta^{15}N$ within populations without comparing to baseline nitrogen values.

Stable isotope analysis of an organism's tissue provides information on diet that is assimilated, not just ingested, over time prior to capture. Tissues have different turnover rates and are therefore useful in determining diet over different temporal scales. Liver has a very high turnover rate of a few weeks (Logan et al. 2006)

whereas scales have a much slower rate and provide information on the diet over a fish's lifetime (Wainright et al. 1993). White muscle has a turnover rate of approximately six months so provides information on diet up to six months prior to capture. This is perhaps the most useful tissue for ecological studies as it would tend to avoid any ontogenetic diet shifts or seasonal variation that scales or liver would incorporate respectively. White muscle is also less variable in C and N than other tissues such as red muscle or liver (Pinnegar and Polunin 1999). There is also considerable variation in isotopic ratios associated with the proportions of dietary constituents, in particular lipids which are known to be $\delta^{13}C$-depleted relative to other tissue constituents such as proteins or carbohydrates (DeNiro and Epstein 1978). This variation can potentially complicate the interpretation of dietary sources of carbon. Models for lipid-normalising $\delta^{13}C$ values from aquatic organisms using C:N ratios have been developed (Kiljunen et al. 2006).

Isotope ratios are expressed conventionally as values in parts per thousand (‰) differences from a standard reference material according to the equation:

$$X = 10^3(R_{sample} - R_{standard}) R^{-1}_{standard}$$

where X is the $\delta^{13}C$ and $\delta^{15}N$ and R_{sample} is the corresponding ratio $^{13}C:^{12}C$ or $^{15}N:^{14}N$. $R_{standard}$ for $\delta^{13}C$ is Vienna PeeDee Belemnite (vPDB) and $R_{standard}$ for $\delta^{15}N$ is atmospheric nitrogen.

2.1.3. Chapter aims

The population of Arctic charr in Windermere, England has been affected in recent years. It is suspected that the amount of habitat available for use by charr has been greatly reduced by the hypoxia of bottom waters and increased temperature of surface waters particularly in the summer months. The reduction in habitat may be exacerbated by increased numbers of roach utilising the pelagic zone. Interspecific competition plays an important role in divergent selection and the formation of divergent phenotypes in many species of postglacial fish, including Arctic charr. Given the plastic nature of Arctic charr, it is hypothesised that the charr will alter their feeding behaviour in order to utilise alternative available habitats. Here, stable

isotope and gut content analysis are used to examine in detail the possible long-term changes in feeding strategy of the Arctic charr population in Windermere in light of these recent environmental changes.

2.2. Methods

2.2.1. Study site

Windermere is situated (54° 22' N, 2° 56' W; altitude 39 m) in the English Lake District, U.K. (Figure 2.1). It was formed in a glaciated valley, approximately 12,000 years ago, and is the largest natural lake in England. The lake comprises a mesotrophic north basin (area 8.1 km^2, maximum depth 64 m) and a eutrophic south basin (area 6.7 km^2, maximum depth 44 m), divided by a shallow sill at a depth of 10 m. The two basins differ in their inflow and outflow characteristics (Elliott and Baroudy 1995). The south basin has a smaller volume and a larger input of streams than the north basin. The water retention time of the whole lake is approximately 9 months (Sutcliffe and Carrick 1983). The Rivers Brathay, Rothay, Troutbeck, and Cunsey Beck as well as many smaller streams feed the basins and water exits the lake via the River Leven, which flows for 3 km into the Irish Sea.

Windermere has resident populations of Arctic charr, brown trout (*Salmo trutta*), perch, pike (*Esox lucius*), European eel (*Anguilla anguilla*) and in recent years, roach. Anadromous brown trout and Atlantic salmon (*Salmo salar*) also pass through the lake as smolts and adults returning to spawn. Several minor species are found in the lake's littoral zone, including stickleback (*Gasterosteus aculeatus*), bullhead (*Cottus gobia*), stoneloach (*Barbatula barbatula*), minnow (*Phoxinus phoxinus*), rudd (*Scardinius erythrophthalmus*) and tench (*Tinca tinca*) (Pickering and Sutcliffe 2001). Historically, Arctic charr have dominated, in terms of biomass, offshore habitats outside of the spawning season (Frost 1965) whereas perch have dominated inshore habitats (Winfield 2004). Contrasting parasite burdens and growth rates of Arctic charr from the two basins (Mills 1989) suggest little movement between them and thus the charr have been proposed to be, and studied as two separate populations.

2.2.2. Sample Collection

Gill netting to capture fish for dietary, morphometric (Chapters Three and Four) and genetic (Chapters Four and Five) analysis was undertaken using single-mesh multifilament gill nets. The nets were set on the lake bottom perpendicular to the shore, and were 1.5 m deep and 30 m long with a single mesh of 33 mm. Nets were set at various sites in both the north and south basins over a period of 7 days from the 17th September 2005 to the 23rd September 2005. A total of 37 Arctic charr were caught from the north basin (mean Fork Length = 253 mm, range 136-330 mm) and 23 Arctic charr were caught from the south basin (mean Fork Length = 264 mm, range 130-322 mm). Benthic gill netting of spawning fish was undertaken by CEH Lancaster as part of a long-term population study. The samples here were caught during the spawning periods, in the months of November (autumn-spawning), and late February and March (spring-spawning) between 2004 and 2006. Spring spawning sites sampled were Holbeck and Rough Holme (north basin) and Rawlinson's Nab (south basin) and autumn spawning sites sampled were High Wray Bay, North Thompson Holme and Rough Holme (north basin) and Grass Holme and Sewage Works (south) (Figure 2.1.). The numbers of fish caught from each site are shown in Table 2.2. In both cases the nets were set late afternoon or early evening and lifted the following morning. All fish were removed from the nets, killed and frozen at –20 °C within 6 hours of capture. A further 29 stomach samples, 23 from the north basin (mean FL = 255 mm, range FL = 241-304 mm) and 6 from the south basin (mean FL = 294 mm, range FL = 254-317 mm), caught using plumb-line in June 2006, were obtained from the Windermere Angling Association.

Table 2.2. Summarised information on the numbers of fish caught from each spawning site in Windermere

Site Name	Basin	Spawning Period	Sex		Total	Mean FL (mm)	Range FL (mm)
			Male	Female			
Rawlinson's Nab	South	Spring	7	21	28	276	248-330
Holbeck	North	Spring	19	11	43	270	244-315
Rough Holme	North	Spring	7	6			
Grass Holme	South	Autumn	9	9	31	274	243-322
Sewage Works	South	Autumn	6	7			
High Wray Bay	North	Autumn	6	2	25	259	215-306
North Thompson Holme	North	Autumn	11	0			
Rough Holme	North	Autumn	4	2			

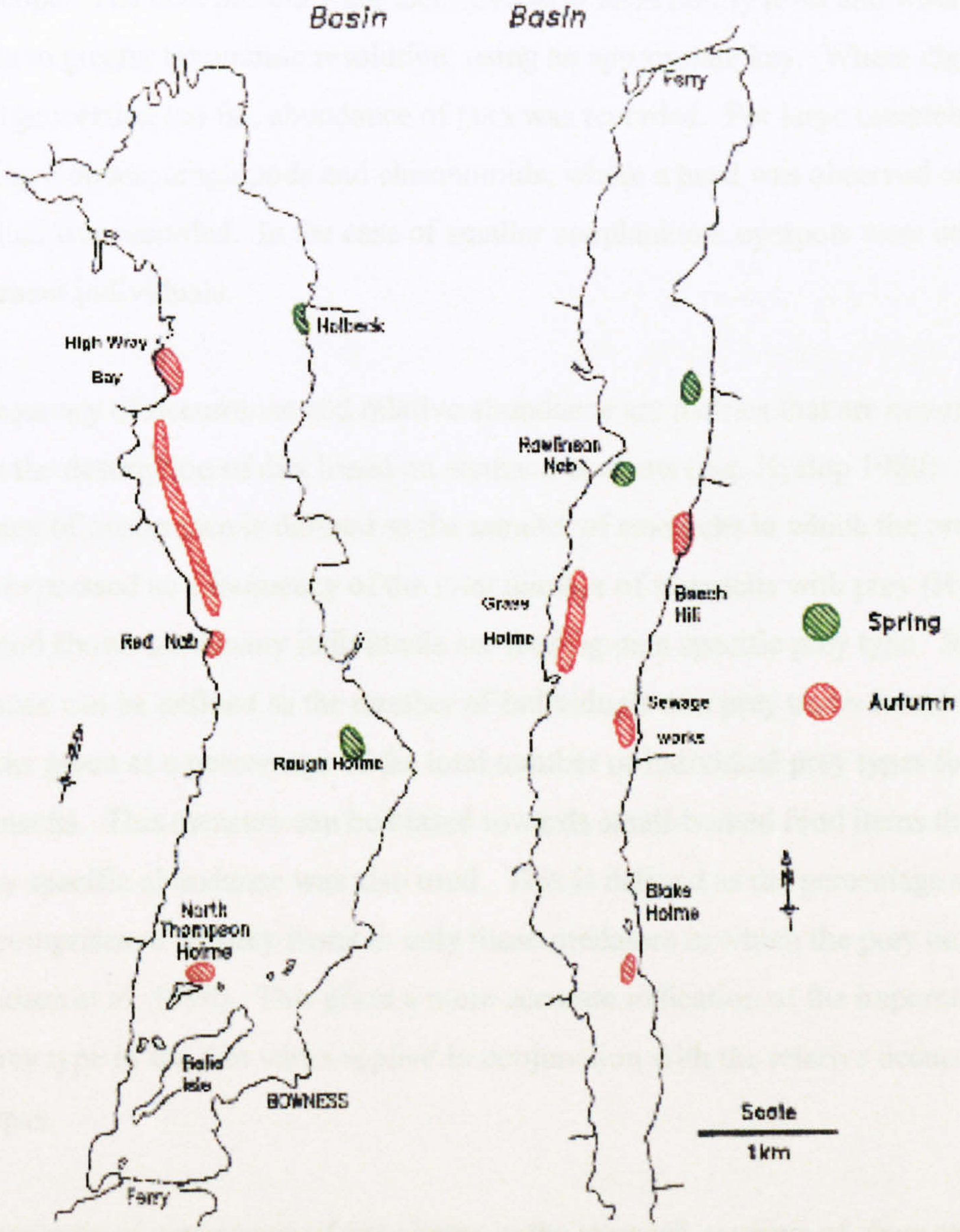


Figure 2.1. The historical distribution of autumn and spring spawning grounds of Arctic charr in Windermere, UK adapted from Frost (1965)

2.2.3. Dietary Analysis

The guts were removed from each fish using incisions at the oesophagus and just before the anus and were preserved in 4 % formalin. Contents were later removed from the stomach, placed in a plastic Petri dish and examined under a dissecting microscope. The taxa present were identified to at least family level and where possible to greater taxonomic resolution, using an appropriate key. Where digestion had not proceeded too far, abundance of taxa was recorded. For large invertebrates, including isopods, amphipods and chironomids, where a head was observed one individual was recorded. In the case of smaller zooplankton, eyespots were counted to represent individuals.

The frequency of occurrence and relative abundance are metrics that are commonly used in the description of diet based on stomach contents (e.g. Hyslop 1980). The frequency of occurrence is defined as the number of stomachs in which the prey occurs expressed as a frequency of the total number of stomachs with prey (Hyslop 1980) and shows how many individuals are feeding on a specific prey type. Relative abundance can be defined as the number of individuals of a prey taxon found in stomachs given as a percentage of the total number of individual prey types found in all stomachs. This measure can be biased towards small-bodied food items therefore the prey specific abundance was also used. This is defined as the percentage a prey taxon comprises of all prey items in only those predators in which the prey occurs (Amundsen et al. 1996). This gives a more accurate indication of the importance of each prey type in the diet when applied in conjunction with the relative occurrence of prey types.

The frequency of occurrence of prey items in the stomach contents of charr caught in the months of March, September and November in gill nets from the present study was directly compared to the frequency of occurrence of prey items from the stomach contents of charr from the same months that are presented in Frost (1977). A comparison was also made between the frequency of occurrence data from angled fish in this study caught in June with that of Frost (1977) for the same month. The

significance of any differences in these values between the two time periods was tested using *r* x *c* contingency tables for each month.

2.2.4. Stable isotope analysis

Fish eat relatively little food during the spawning period; therefore gut contents of these fish gave limited information on their feeding habits. In order to obtain information on the diet of spawning fish stable isotope analysis was done on samples of their white muscle tissue. Due to limited resources stable isotope analysis could only be done on a subset of ten individuals from both autumn and spring spawning populations from the north and south basins. Nevertheless this sample size is similar to those used in other studies (McCarthy et al. 2004). Stable isotope analysis was also carried out on a sub set of eight charr caught using benthic gill nets from the South basin during September 2005. Unfortunately no muscle tissue samples were available for angler-caught fish so these could not be analysed by stable isotope methods.

Muscle tissue was removed from just below the dorsal fin of fish to be analysed, placed in a 1.5 ml universal tube and frozen at -20 °C for storage. The muscle tissue was then defrosted prior to being dried at 60 °C in a drying oven for 48 hours. The sample was then ground into a fine powder using a pestle and mortar. The pestle and mortar were washed, dried and disinfected with 70 % ethanol after each sample to avoid contamination. Approximately 0.4 mg of each sample was weighed out and loaded into 4 x 6 mm tin capsules and combusted in a Carlo Erba C/N/S analyser, interfaced with a continuous flow isotope ratio, mass spectrometer. A previous study has shown that repeat analysis in white muscle samples results in sample reproducibility close to unity (McCarthy et al. 2004), therefore single measurements were made for all fish samples. $\delta^{13}C$ values were lipid normalised prior to interpretation using the following equations:

$$L = \frac{93}{1 + (0.246 \times (C:N) - 0.775)^{-1}}$$

$$\delta^{13}C' = \delta^{13}C + D \times \left(I + \frac{3.90}{1 + 287/L} \right) \qquad 26$$

Where L is the proportional lipid content of the sample and $\delta^{13}C'$ is the lipid normalised value of the sample; C and N are the proportions of carbon and nitrogen in the sample; $\delta^{13}C$ is the measured value of the sample; *D* is the isotopic difference between protein and lipid in the tissue and has been assigned a value of 7 based on literature data; and *I* is a constant (assigned a value of 0.048) (Kiljunen et al. 2006).

Fresh samples of macroinvertebrate and zooplankton prey species were also included in the stable isotope analysis in order to compare carbon and nitrogen ratios with those of the fish. The benthic macroinvertebrates were captured by kick sampling in the littoral zone in both basins and zooplankton was obtained using zooplankton nets trawled over the deepest points in both basins. These invertebrates were sorted to genus level and in order to obtain enough dry matter for the analysis, several specimens were combined in tubes prior to drying at 60 °C as above.

2.3. Results

2.3.1. Dietary analysis

Prey types consumed by Arctic charr in Windermere can be divided into three principal groups; pelagic zooplankton, including the cladocerans, *Daphnia* and *Leptodora,* and *Chaoborus* larvae; benthic macroinvertebrates, including *Asellus*, *Gammarus*, and chironomid larvae; and fish. The frequency of occurrence, relative abundance and prey-specific abundance for each prey type is displayed in Tables 2.3 and 2.4.

Table 2.3. Relative abundance (percentage of food items) and frequency of occurrence (percentage of total fish containing food, given in parenthesis) of prey types in the diet of Arctic charr caught using benthic gill nets in Windermere from 2003-2007. The food taxa are ordered by percentage of the total frequency containing each food item.

Month	March		September		November		Total frequency of occurrence		Percentage	
	North	South	North	South	North	South	North	South	of total	Total prey specific
No. fish examined	43	28	37	23	25	31	105	82	**frequency**	**abundance**
No. fish feeding	1	17	34	16	2	0	37	33		
Chironomid larvae		3.0 (23.5)	73.3 (88.2)	74.7 (100.0)	10.0 (50.0)		78.4	60.6	70.0	67.9
Asellus		73.8 (94.1)	2.2 (41.2)	8.3 (68.8)			40.5	81.8	60.0	17.7
Gammarus		19.0 (47.1)	0.6 (17.6)	0.3 (12.3)			16.2	30.3	22.9	10.4
Chironomid pupae			19.6 (44.1)	10.5 (6.3)	40.0 (50.0)		37.8	3.0	21.4	54.2
Leptodora			10.0 (11.8)	0.3 (6.3)			10.8	3.0	7.1	40.9
Fish			0.3 (11.8)				10.8	0	5.7	14.3
***Chaoborus* larvae and pupae**		3.4 (5.9)		5.8 (12.3)			0	9.1	4.3	57.0
Charr eggs	100 (*100*)	0.8 (5.9)					2.7	3.0	2.9	100.0
Daphnia					50.0 (50.0)		2.7	0	1.4	80.0
Pisidium				0.07 (6.3)			0	3.0	1.4	1.6

Table 2.4. Relative abundance (% of food items) and frequency of occurrence (% of fish containing food, given in parenthesis) of prey types in the diet of Arctic charr caught by plumb line in the north (*n*= 23) and south (*n*=3) basins of Windermere, June 2006. Prey categories are ordered according to the percentage of the total frequency.

Prey Items	North basin	South basin	Percentage of total frequency	Total prey specific abundance
Chironomid larvae	44.4 (95.6)	19.1 (66.7)	92.3	44.0
Leptodora	48.7 (39.1)		34.6	71.9
***Chaoborus* larvae and pupae**	3.2 (27.3)	77.6 (66.7)	30.8	19.6
Asellus	1.1 (21.7)	1.4 (33.3)	23.1	6.6
Fish	0.1 (13.1)		11.5	23.1
Gammarus	0.9 (13.0)		11.5	6.8
Daphnia	0.8 (8.7)		7.7	7.8

The composition of diet varied in fish caught at different times of the year. In spring, charr caught using benthic nets were consuming a large proportion of benthic macroinvertebrates. *Asellus* and *Gammarus* were most important, occurring in the majority of individuals in high numbers. Planktonic prey was much less important as *Chaoborus* larvae were the only planktonic prey found and this only occurred in one individual in extremely low numbers. Charr eggs were found in stomachs of only two individuals but also in very low numbers. In the late summer, the variety of prey found in the diet of gill-net caught charr increased to include chironomid larvae and pupae, zooplankton and fish fry. Chironomid larvae were by far the most abundant prey category occurring in high numbers, in many individuals. Unfortunately, out of the 56 autumn spawning fish caught only two had stomachs containing prey; therefore little information can be obtained from stomach content analysis for this period of the year.

Overall, the frequency of occurrence of benthic macroinvertebrates such as chironomid larvae, *Asellus* and *Gammarus*, was high (70%, 60% and 23% of fish

respectively) in fish caught in benthic nets whereas the occurrence of zooplankton prey was much lower (<10% of fish). The total prey specific abundance of different prey types indicates that benthic macroinvertebrates were highly abundant in stomach contents across both spring and late summer samples. Planktonic prey species also had high prey specific abundance indicating that they occurred in high numbers in those few individuals in which they were present.

The frequency of occurrence of benthic macroinvertebrates was also very high in those fish caught in June by plumb line, with 92 % of fish containing chironomid larvae and 23 % of fish containing *Asellus*. *Chaoborus* was a very important food category with a relative abundance of 78 % in those fish from the south basin but was only found in 30.8 % of all fish containing food. *Leptodora* also occurred in high numbers with a relative abundance of 49 % in 39 % of fish, all from the north basin. All other prey types had relative abundances of < 10 % (Table 2.4).

Comparisons between the frequencies of occurrence of prey items found in the stomachs of charr in the present study and those of charr from 1940-1951, published by Frost (1977) highlight significant changes in dietary composition between the two time periods (March: $\chi^2 = 205.2$, $P < 0.001$; June: $\chi^2 = 171.3$, $P < 0.001$; September: $\chi^2 = 236.5$, $P < 0.001$; November: $\chi^2 = 125.3$, $P < 0.001$). Based upon data from plumb-line methods Frost (1977) reported that during the years 1940-1951, in June zooplankton species had a frequency of occurrence value of 40 % for *Daphnia* and 53 % for *Leptodora* compared 8 % frequency for *Daphnia* and 35 % for *Leptodora* in the present study (Table 2.5).

Table 2.5 shows that for November the frequency of occurrence of *Leptodora* was 50 % in the present study; higher than the 19 % found by Frost (1977), however this is based solely on the total of two fish out of the 58 caught during Autumn spawning period that had stomach contents compared to the 43 feeding fish used for Frost's (1977) analysis. The occurrence of *Leptodora* and *Daphnia* in the diet of fish caught in June 2006 by plumb-line was greater than that of any other month over the same period but was not as high as in the previous study. Along with this change, a greater

incidence of benthic invertebrates in charr diet can be seen now compared to 1940-1951. Over the months that can be compared to the present study, chironomid larvae were found in relatively few fish compared to the present findings, for example, when at their highest frequency in the previous study in March, were present in only 10 % of charr stomachs compared to 22 % of stomachs in the present study. Also, in the present study they were found in the majority of stomachs in September, where they were found in 88 % of stomachs, compared to 7 % in the previous study. *Asellus*, present in 89 % of charr caught in March and 52 % in September of the present study are not mentioned at all in Frost's (1977) study.

Table 2.5. A comparison of the frequency of occurrence (percentage of feeding fish in which they occur) of prey items in the diet of Arctic charr in Windermere (North and South basins combined) over the periods 2003-2007 (the present study; fish caught by gill net and plumb-line) and 1940-1951 (Frost, 1977; fish caught by gill net and plumb line) (n = the number of feeding fish)

Month	**March**		**June**		**September**		**November**	
Year	1940-1951	2003-2007	1940-1951	2006	1940-1951	2003-2007	1940-1951	2003-2007
	n = 50	*n* = 18	*n* = 38	*n* = 26	*n* = 27	*n* = 50	*n* = 43	*n* = 2
Method of capture	Gill-net	Gill-net	Plumb-line	Plumb-line	Plumb-line	Gill-net	Gill-net	Gill-net
Chironomid larvae	10	22	37	92	7	88		50
Chironomid pupae	28		60			30		50
***Chaoborus* larvae and pupae**		6	24	31	15	4		
Asellus		89		23		52		
Gammarus	2	44		12	4	12	2	
Leptodora			53	35	55	10	19	50
Daphnia	2		40	8	51		28	
Pisidium	2					2	5	
Fish			-	12		8		
Charr eggs	68	11					51	

2.3.2. Stable isotope analysis

Lipid normalised carbon and nitrogen stable isotope values of muscle from charr caught in benthic gill nets in Windermere varied between individuals from –22.97 to -27.06 ‰ and from 10.05 to 15.32‰ respectively. Both mean nitrogen and lipid normalised carbon stable isotope values differed significantly between spawning populations (one-way ANOVA: $F_{3/33} = 35.97$, $P < 0.0001$, $F_{3/33} = 14.24$, $P < 0.0001$, respectively) and the Bonferroni-corrected significance levels between pairs of spawning populations are presented in Table 2.6. The south basin autumn and spring spawning groups were significantly less depleted in mean $\delta^{13}C$ than the north basin autumn and spring spawning groups (South basin mean $\delta^{13}C = -24.44$ and –24.38 ‰ respectively *c.f.* –26.55 and –25.82 ‰ from the North basin) (Figure 2.2). The south basin autumn and spring spawning groups also had significantly higher mean $\delta^{15}N$ values than both north basin spawning groups (14.61 and 13.82 ‰ *c.f.* 12.59 and 11.24 ‰ respectively) but in this case the spring spawning group from the north basin also had a significantly higher mean $\delta^{15}N$ value than the autumn spawning group from the same basin. The summer group, caught in September, from the south basin was similar in mean $\delta^{15}N$ value to the other south basin groups but was significantly less depleted in mean $\delta^{13}C$ than the north basin autumn spawning group only.

Table 2.6. Bonferroni corrected significance levels from one-way analysis of variance of nitrogen (above the diagonal) and lipid normalised carbon (below the diagonal) stable isotope ratios between pairs of populations of Arctic charr from Windermere (AS, autumn south, AN, autumn north, SS, spring south, SN, spring north, SSU, summer south).

	AS	AN	SS	SN	SSU
AS		0.0001	n.s.	0.001	n.s.
AN	0.004		0.0001	0.01	0.0001
SS	n.s.	0.0001		0.01	n.s.
SN	0.01	n.s.	0.01		0.001
SSU	n.s.	0.01	n.s.	n.s.	

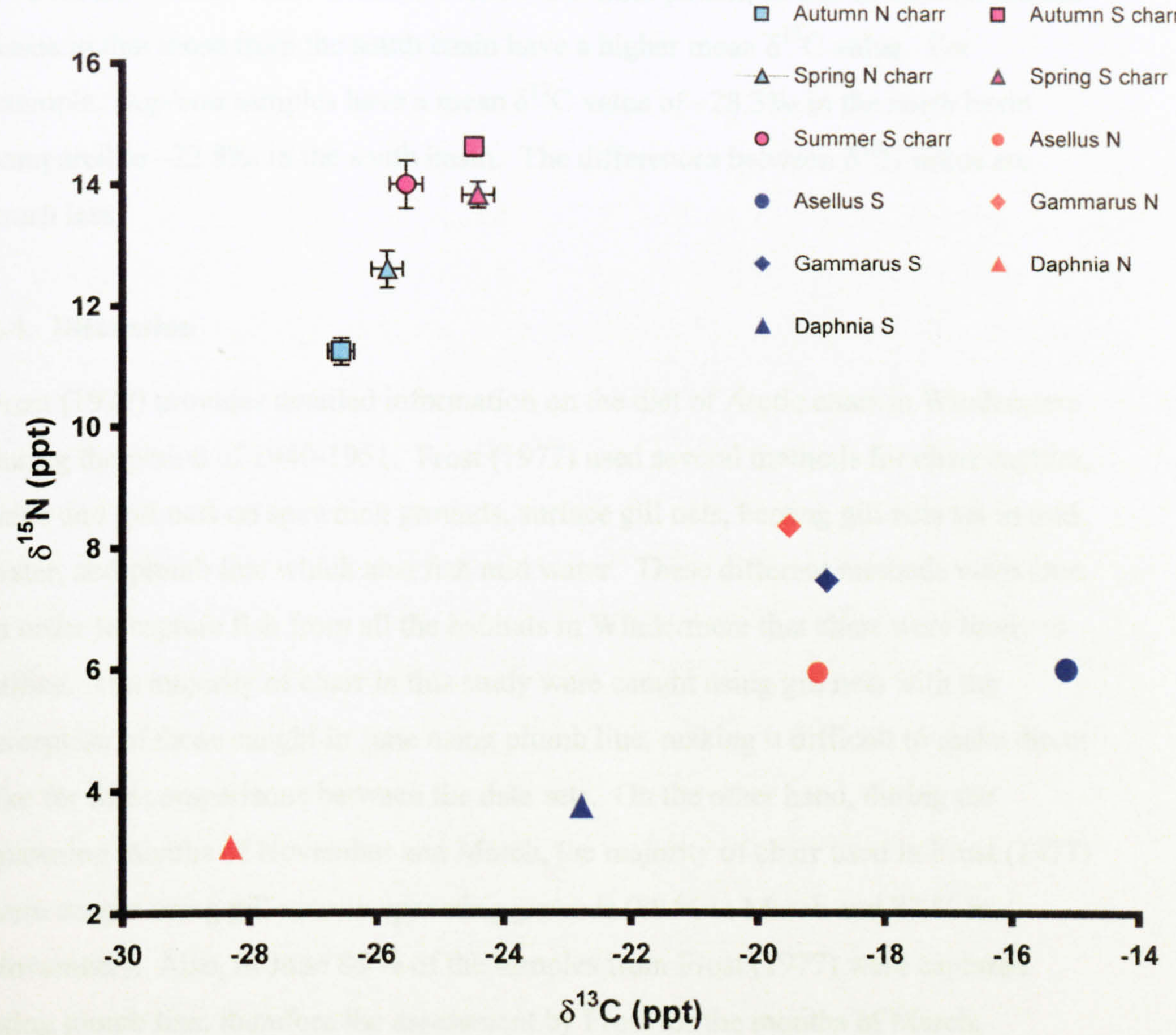


Figure 2.2. The mean ± S.E. $\delta^{15}N$ and lipid normalised $\delta^{13}C$ in the white muscle in Arctic charr collected from spawning grounds in the north and south basins during spawning periods, and charr collected in September from the south basin, and the mean ± S.E. $\delta^{15}N$ and $\delta^{13}C$ of invertebrate prey items collected from the north and south basins of Windermere.

Figure 2.2 also presents the stable isotope signatures of invertebrate prey taxa from both the north and south basins of Windermere. Benthic macroinvertebrates, including isopods e.g. *Asellus* and amphipods e.g. *Gammarus* exhibited similar stable

isotopic values of approximately –16.00 ‰ to –20.00 ‰ for $\delta^{13}C$ and between 5 and 8 ‰ for $\delta^{15}N$. These values differ greatly from those of *Daphnia*, which were much more depleted in $\delta^{13}C$ and $\delta^{15}N$ especially in the North basin. The differences in $\delta^{13}C$ isotopic ratios of invertebrates follow a similar pattern to that of the fish muscle tissue in that those from the south basin have a higher mean $\delta^{13}C$ value. For example, *Daphnia* samples have a mean $\delta^{13}C$ value of –28.3‰ in the north basin compared to –22.8‰ in the south basin. The differences between $\delta^{15}N$ ratios are much less.

2.4. Discussion

Frost (1977) provides detailed information on the diet of Arctic charr in Windermere during the period of 1940-1951. Frost (1977) used several methods for charr capture, seine and gill nets on spawning grounds, surface gill nets, herring gill nets set in mid water, and plumb line which also fish mid water. These different methods were used in order to capture fish from all the habitats in Windermere that charr were likely to utilise. The majority of charr in this study were caught using gill nets with the exception of those caught in June using plumb line, making it difficult to make direct like for like comparisons between the data sets. On the other hand, during the spawning months of November and March, the majority of charr used in Frost (1977) were caught using gill nets on spawning grounds (80 % in March and 87 % in November). Also, in June 86 % of the samples from Frost (1977) were captured using plumb line, therefore the assessment by Frost for the months of March, November and June are fairly analogous to the present study. September is the only month where the methods of capture are completely different, gill nets in this study and mostly plumb line in Frost (1977). Despite these differences and fewer samples for fish caught on spawning grounds in March and November in the present study, comparisons of the frequency of occurrence of different prey types can be made between the present diet and that of around 60 years ago, prior to the environmental problems apparent in Windermere over the past decades.

Arctic charr in Windermere have been previously described as zooplanktivorous for the most part of the year (Frost 1977). When the abundance of zooplankton

decreased during autumn, charr diet consistently had a higher proportion of benthic invertebrates. An offshore movement during the summer months when zooplankton are abundant is commonly seen in Arctic charr (Hindar and Jonsson 1982b, Langeland et al. 1991, Amundsen 1995) as well as other temperate freshwater species (Werner and Hall 1976, Mittelbach 1981).

The results of the present study show a change in this behaviour. Dietary analysis shows a much lower frequency of occurrence of zooplankton prey being consumed by charr now than previously (Table 2.5). Those charr caught by plumb line from both basins in June, which may have been expected to be consuming mostly pelagic prey, contained high numbers of benthic prey items such as chironomid larvae and *Asellus* as well as low numbers of zooplankton prey (Table 2.4). However, relatively large abundances of *Chaoborus* were also found in the diet of charr caught mid water. *Chaoborus* undergoes daily migrations through the water column inhabiting the benthos throughout the day and ascending to the surface to feed at night. Therefore charr could be feeding on them on the benthos or in the water column. In comparison with the dietary data from Frost (1977) from the same month, fewer charr caught by plumb line in this study were consuming pelagic prey items relative to 60 years ago. Chironomid pupae were fairly important in the diet of charr in November but this was based on few fish whereas during June they had high frequency of occurrence in both studies. Frost (1977) argued that although chironomid larvae inhabit deep sediments, their pupae rise to the surface prior to their emergence and were therefore preyed upon by charr in the water column. Although this may be true, the majority of the life cycle is spent within the sediment including the pupating stage and it is likely that the pupae only occupy the water column for a short period during their ascent. This usually occurs in May or June so it is not surprising that chironomid pupae form part of the diet during this time of year but it is unclear if they are consumed in the water column or from the benthos (Willoughby 1976).

The stable isotope technique provided an assessment of assimilated food intake over a significantly longer period than stomach contents analysis. Diet is identifiable as

the carbon stable isotope signature of a predator is similar to the signature of its prey whereas the nitrogen stable isotope signature will be enriched 3-4 ‰ compared to the signature of prey. Both mean carbon and nitrogen stable isotope ratios of charr from both basins of Windermere are similar to those found from charr in other lakes. Adams et al. (2003) recorded $\delta^{13}C$ values of between –29.1 and –24.4 ‰ and $\delta^{15}N$ values of 8.6 to 14.0 ‰ in Arctic charr from Loch Tay in Scotland. The significant differences in isotopic ratios between fish caught from the two basins could be due to a number of factors. It could be caused by a difference in diet although this is unlikely as the patterns between basins are reflected in the isotopic values of prey items also. It is far more likely that the difference is caused by differences in the water chemistry of the basins or differences in depth. The $\delta^{13}C$ values of both particulate organic matter (POM) and sediments do not differ between basins (J. Grey, pers. com.) suggesting that baseline carbon values are similar in each basin. There is however $\delta^{15}N$ enrichment of POM in the south basin relative to the north basin across seasons (south basin $\delta^{15}N = 5.0$ *cf.* north basin $\delta^{15}N = 4.3$ in the spring; south basin $\delta^{15}N = 8.0$ *cf.* north basin $\delta^{15}N = 5.0$ in the summer) suggesting differences in baseline nitrogen values between the basins (J. Grey, pers. com). Carbon and nitrogen stable isotope ratios also both decrease with increasing depth (Grey 2006). The north basin has a greater mean and maximum depth than the south basin. Charr feeding on the benthos would therefore feed at a greater average depth in the north basin compared to in the south basin. It is therefore not surprising that charr from the north basin to have reduced carbon and nitrogen stable isotope ratios. When compared to those of the prey types, the $\delta^{13}C$ signatures of the fish in the North basin indicate that they neither feed solely on zooplankton or benthic macroinvertebrates as their values lie between the mean values for these two types of prey. The mean $\delta^{13}C$ signatures of spawning groups in the south basin, on the other hand, is less enriched compared to the zooplankton carbon isotope signature for the south basin (Figure 2.2) but the mean $\delta^{15}N$ is too enriched for the charr to be feeding solely on herbivorous zooplankton such as *Daphnia* and probably also for predatory zooplankton such as *Leptodora*. Chironomid larvae are highly abundant in the stomach contents but attempts to collect samples for stable isotope analysis were unsuccessful. Other studies, however, have shown them to have much depleted $\delta^{13}C$

signatures of between –28 and –70 ‰ depending on the depth at which they occur and the rates of methane turnover of sediments (Deines et al. 2007). Data from Windermere showed the mean carbon stable isotopic values of the chironomid genus, *Sergentia*, measured in the spring, to range from –37.8 ‰ in the north basin to –31.3 ‰ in the south basin in the spring and from –34.4 ‰ in the north basin to –29.8 ‰ in the south basin in the summer (J. Grey pers. comm.). It is therefore possible that consumption of chironomid larvae will reduce the carbon stable isotope signature of charr in both basins beyond that of a diet solely made up of other benthic macroinvertebrates.

The values of $\delta^{15}N$ of a predator are generally 3-4 ‰ greater than that of their prey. The values of charr in Windermere are comparable to charr with benthic signatures elsewhere (Guiguer et al. 2002). The $\delta^{15}N$ values of charr from both basins are enriched by approximately 7-10 ‰ compared to that of zooplankton, whereas the charr are less enriched compared to the benthic invertebrates (a value of approximately 4-6 ‰), suggesting the diet has a benthic source.

The stomach contents analysis shows a marked increase in the abundance of benthic invertebrates in the present diet and, whilst zooplankton prey types are present, they occur in much lower abundances than they did previously. Although the dietary analysis is limited by a lack of feeding specimens during the autumn spawning period, it is reinforced by the stable isotope data, which indicates that Windermere charr are not feeding predominantly on zooplankton as previously found. There are several possible explanations for these observations; competition by the expanding roach population, eutrophication, increased surface temperatures or a combination of all factors is forcing Arctic charr into an alternative niche (Winfield et al. 2007).

There is good evidence that food limits whole fish communities and that fish partition resources according to their competitive capabilities (Nilsson 1963, Hindar et al. 1988, Langeland et al. 1991, Blackie et al. 2003, Amundsen et al. 2004). Field studies of competitive interactions often base their conclusions on comparisons of populations of two species occurring in sympatry with conspecific populations

occurring in allopatry (e.g. Nilsson 1963). Windermere provides a unique opportunity to assess the effects of possible competitive interactions between Arctic charr and roach within the same lake by comparing present data with data that exists prior to the roach population expansion. Habitat segregation is a common outcome of competitive interactions among fish species (see Chapter One). It is well documented from field studies of Arctic charr and similar species e.g. Dolly Varden charr, that allopatric and sympatric populations of the same species differ in their diet and spatial distribution (Skúlason and Smith 1995). Those species that show habitat segregation in sympatry will often expand their niches in allopatry, a process known as competitive release (e.g.Schluter and McPhail 1993, Robinson and Wilson 1994).

Differences in aggressive behaviours are often the primary factors instigating habitat or resource segregation between competing species, with the subordinate species being the more generalistic feeder (Larson 1980). Laboratory studies of Dolly Varden charr and its competitor, cutthroat trout (*Oncorhynchus clarki*), which occur in British Columbia, indicate that cutthroat trout are much more aggressive and will dominate charr when sharing aquaria (Hindar et al. 1988). Dolly Varden charr are also more flexible in their foraging behaviour. They are able to locate prey at much lower light intensities than the trout and can detect buried prey (Jobling et al. 1993). They are therefore able to avoid the aggressive behaviour of the trout by moving to deeper water and switching to a benthic diet (Hindar et al. 1988). The generalist diet of Arctic charr is well documented and laboratory studies show that they are able to switch prey types depending on the availability of prey (Amundsen 1995). Charr are also able to locate prey at low light intensities (0.0003-0.003 W m^{-2}) (Jorgensen and Jobling 1990), as well as buried prey, and therefore are easily able to utilise benthic resources. It is therefore not inconceivable that charr may increase their utilisation of benthic habitats in the face of competitive interactions with a zooplanktivore.

Studies assessing the competitor interactions between roach and fish species such as perch, have shown that roach is the superior competitor in open water habitats dominated by zooplankton. Roach are known to be non-aggressive fish species therefore competition is by exploitative competition, in which one species reduces

the availability of a resource to levels that cannot be utilised by the other species (MacKenzie et al. 1998). Roach are highly efficient zooplankton feeders (Langeland and Nost 1995) and have high a high attack rate and lower handling times when feeding on zooplankton than benthic macroinvertebrates such as chironomid larvae (Persson 1987). Roach also have a high swimming speed of 0.5 m s^{-1} allowing high foraging efficiency on zooplankton in the pelagic zone.

Arctic charr and roach rarely occur sympatrically and there are no experimental studies that have addressed the competitive interactions between them. There is however a study of the affects of roach introduction on whitefish (*Coregonus lavaretus*) in lake Haukvatn, Norway, a species with quite similar requirements to charr (Langeland and Nost 1994). Both species are considered planktivores and would thus utilise the same resources. The catch of both young and adult whitefish was greatly reduced after the roach population had become established. Therefore Langland and Nost (1994) hypothesised that competition between roach and young whitefish caused a bottleneck for recruitment to the adult whitefish population.

Despite the lack of experimental evidence of direct competitive interactions between roach and Arctic charr, the reduction in numbers of Arctic charr in large lakes in West Ireland have been closely associated with increased numbers of roach (Igoe et al. 2001, Igoe and Hammar 2004). Igoe et al. (2004) reviewed data on Arctic charr population status in Irish lakes and found that significantly more lakes where charr populations became extinct also contained non-indigenous species. Observational data suggest that reductions of Arctic charr catches in Windermere are also closely associated with rises in roach catches. Winfield et al. (2007) recorded the species composition caught in nets set in both off shore surface (0-1m deep) and offshore bottom waters of the North (60 m deep) and South (40 m deep) basins of Windermere from 2001 to 2004. In the North basin, Arctic charr dominated both of these habitats in 2001 and 2002, with abundances over 90 %. By 2003, however, they continued to dominate the bottom waters but their abundance had reduced to 40 % in the surface water catches, and by 2004, this value had lowered to 10 %. The catches in 2003 consisted mainly of perch and brown trout whereas the catches in

2004 consisted mainly of perch and roach. The offshore surface catches from the South basin had fewer charr than the North basin throughout the years sampled, ranging from < 30 % in 2001 to < 10 % in 2004; the majority of these catches consisting of roach and perch. Arctic charr were more abundant in the catches from the bottom waters of the south basin, ranging from 90 % in 2001 to 50 % for 2003 (no data was available for 2004). Although inconclusive, the reduction in catches of Arctic charr in surface nets may also evidence a competitive interaction between roach and charr.

Temperature may also influence the diet of Arctic charr by affecting their vertical distribution in the water column. The optimum temperature range for growth of parr and adult charr is 11-14 °C but they can feed and grow at temperatures as low as 1-3 °C (Larsson et al. 2005). As surface water temperatures of Windermere have increased over recent years (Winfield et al. 2004), it is expected that charr may be forced to forage in deeper areas. However, a recent study using hydroacoustic surveys and oxygen and temperature profiles, in order to assess habitat availability in Windermere from 2002 to 2004 (Jones et al. 2008), found that the 12 °C isotherm is shallow enough (< 20m) to allow movement of Arctic charr up the water column in late summer, but that they rarely cross the 16 °C isotherm that can extend over 10m deep in late summer.

Changes in diet composition can also be caused by changes in the availability of different prey species. Unfortunately it was not possible to assess the potential food resources in Windermere but there is no evidence to suggest a change in the abundance of zooplankton since Frost's study. In fact, any change caused by increased nutrient load or surface temperature, is likely to increase rather than decrease the abundance of zooplankton (Parker and Maberly 2000). It is possible however, that increases in lake surface temperature may affect mixing regimes, altering phytoplankton development and community composition. This may consequently affect the community composition of the zooplankton (George et al. 2004). This seems unlikely however as a reduction in the abundance of appropriate

zooplankton prey would also have negatively impacted the roach population whereas the opposite appears to be true (Winfield et al. 2006).

The data presented indicate that the diet of Windermere Arctic charr has changed since Frost's (1977) study, reflecting environmental change in the lake. The existence of historic datasets such as this is informative for determining ecological responses to environmental change. Niche shifts such as is observed in Windermere charr, are common in Arctic charr faced with interspecific competition (Hindar et al. 1988; Langeland et al. 1991; Forseth et al. 2003). However, the direction of the niche shift is not the same in each case, and is likely to depend on environmental factors such as temperature, resource and habitat availability, as well as the nature of the competitor involved. In this case, increases in surface temperature and exploitation competition by roach may be causing the competitive exclusion of Arctic charr in the pelagic zone. This is encouraging the utilisation of benthic habitats and the consumption of benthic invertebrates such as chironomids, which are highly abundant in their stomach contents throughout the year. The rapid expansion of the roach population in recent years could lead to depletion of zooplankton in summer months but further assessment of the zooplankton abundance relative to other food resources is required.

Chapter 3 : Phenotypic diversity of Arctic charr in populations in the Lake District, UK

3.1. Introduction

3.1.1. Phenotypic variation in Arctic charr

It is well documented that, among the salmonids, Arctic charr exhibits the greatest extremes of phenotypic variability (Klemetsen et al. 2003) and in some cases, morphological and trophic variation between intraspecific morphs may exceed differences between species (Malmquist et al. 1992). Arctic charr illustrate morphs that display variation in body size, migration patterns, head and mouth anatomy, colouration, meristic characters, time of spawning, life history patterns and ecology (Hindar and Jonsson 1993, Jonsson and Jonsson 2001). This variation has been observed between populations in different drainage basins and also in systems where two or more phenotypic variants exist in sympatry (Klemetsen et al. 2002). These morphological differences are clearly correlated with the availability, number and discreteness of habitat and individuals foraging behaviour (Adams et al. 1998, Skulason et al. 1999).

Sympatric morphs of Arctic charr are typically differentiated into a benthic morph and a limnetic morph, named after the habitats in which they commonly occur, but morphs related to size at maturation are also common. For instance, Vangsvatnet Lake in Western Norway supports two charr morphs. These morphs are described in the literature as 'dwarf' and 'normal' charr (Nordeng 1961) but could also be described by their habitat use, as 'littoral' and 'profundal' charr, as has been done in other lakes. The dwarf charr retains its juvenile colouration (Hindar and Jonsson 1982a) and has a lower growth rate and smaller adult size than the normal charr. The morphs also differ in morphology associated with diet. The dwarf charr have a more protruding upper jaw and feeds on zoobenthos in the profundal zone whereas the normal charr feed on zooplankton in the littoral zone. The best described case of sympatric polymorphisms is that of Lake Thingvallavatn, Southwest Iceland, where four morphs occur, two benthic and two limnetic. The benthic morphs both have

blunt snouts and sub-terminal mouths adapted to feed on benthic invertebrates but differ in growth rate and adult size. One, termed, the profundal benthic morph, is found in the deeper profundal zone, below the thermocline, and generally has a slower growth rate and smaller size at maturation; mature females as small as 73 mm have been found. The littoral benthic morph that inhabits the littoral zone has a faster growth rate. The difference in growth rates between these morphs has been attributed to limited food availability in the profundal zone. The limnetic morphs both have pointed snouts and terminal mouths adapted to prey capture in the open water. Again both limnetic morphs differ in size; one morph feeds on zooplankton and has a relatively slower growth rate and smaller size at maturation than the larger piscivorous morph (Snorrason et al. 1994). Lake Thingvallavatn is the only lake where so many morphs are known to occur in sympatry (Skulason et al. 1992) and is also unusually stable, for example diet segregation is maintained throughout the year (Malmquist et al. 1992), whereas in most other cases segregation is more flexible (Skulason et al. 1992). It is unknown why Thingvallavatn in particular maintains such a complex sympatric system, but it is thought that the high physical complexity of the volcanic lake substrate provide increased opportunities for niche expansions (Skulason et al. 1989). Further examples of resource polymorphism in Arctic charr include Loch Ericht and Loch Rannoch, Scotland (Walker and Greer 1988, Adams et al. 1998, Fraser et al. 1998).

Brook charr, a closely related species to Arctic charr, in lakes Bondi and Ledoux, Quebec, Canada, can be divided into two groups based on habitat, a littoral group which inhabit surface waters to 2 m deep, and a profundal group, inhabiting water 3-6 m deep (Dynes et al. 1999). These habitat differences are related to morphological differences that could be expected to maximise foraging ability. The pelagic group has a shorter dorsal fin and longer body length posterior to the dorsal fin than the littoral fish. This minimises drag and allows efficient cruising thus maximising the ability to search and feed in open water. The benthic group has longer pectoral fins (5.2 cm ± 0.5 *cf.* 4.1cm ± 0.5 of pelagic morphs) allowing slow and precise manoeuvring that is required living in structurally complex habitats and for feeding on benthic organisms (Bourke et al. 1997).

Parasitic infestations reflect the long-term feeding behaviour and food web interactions of fish species. It is therefore not surprising that morphs commonly vary in their associated parasite species composition and abundance. In Lake Fjellfrøsvatn, northern Norway, dwarf Arctic charr inhabiting the profundal zone had many fewer parasite species than the 'normal' sized charr inhabiting the littoral (Knudsen et al. 1997). Similarly, in Lake Thingvallavatn, Iceland, differences in parasite species composition were observed in the four morphs (Sandlund et al. 1992). These differences are caused by transmission of different parasites from different prey species.

Arctic charr morphs may also vary in time and location of spawning. Arctic charr usually spawn over a 2-3 week period in autumn so that the rate of egg development results in the young swimming out of the gravel in spring and beginning to feed when food availability is high, however the time of spawning can be earlier or later in habitats less affected by seasons, such as deep water, where temperature is more constant (Johnson 1980, Klemetsen et al. 1997). However, in some lakes an extra spawning season occurs, usually in the spring or summer (Frost 1965, Skulason et al. 1989), and as sexually mature Arctic charr home to their natal grounds, the availability of different spawning habitats can lead to development of distinct spawning habitats among morphs. In Thingvallavatn, the large benthivorous charr spawn in areas of the lake with an inflow of cold groundwater in July and August, whereas the small benthic charr spawn in the shallow littoral from September to November. Both limnetic morphs spawn on stony littoral substrate from September to November (Sandlund et al. 1992). Also, in Vangsvatnet Lake, Norwary, dwarf and normal charr spawn two weeks apart and at different depths (Hindar and Jonsson 1982b).

3.1.2. Mechanisms for morphological diversification

3.1.2.1. Phenotypic plasticity and genetic basis

The causes of much of the phenotypic variation of Arctic charr are commonly attributed to their ability to produce multiple alternative morphologies and/or behaviours in response to environmental conditions, termed phenotypic plasticity

(West-Eberhard 1989). The opportunistic feeding behaviours of charr allow their adaptive expansion into multiple available niches, leading to the development of alternative phenotypes when in sympatry with species with similar requirements (Dynes et al. 1999). Arctic charr tend to be plastic in many phenotypic attributes (e.g.Nordeng 1983, Jobling et al. 1993), but common garden and breeding experiments have also shown a direct genetic basis for phenotypic attributes that differ among morphs (Nordeng 1983, Adams and Huntingford 2002a, Klemetsen et al. 2002).

Using rearing and transplant experiments Klemetsen et al. (2002) demonstrated that offspring from profundal and littoral morphs from Lake Fjellfrosvatn, Norway, maintained the morphology and feeding behaviours of their parents when reared under identical environmental conditions, indicating a genetic basis. Their growth rate, however, altered in the laboratory. The usually slow-growing profundal morph had a much higher growth rate when removed from the restrictions of its unproductive environment. Adams and Huntingford (2002) also found that morphological variation of benthivorous and planktivorous morphs from Loch Rannoch, Scotland, persisted in offspring when reared in identical conditions, concluding that these traits are inherited. The same morphs from Loch Rannoch also show a clear genetic component with respect to differences in social behaviour (Mikheev et al. 1996). Nordeng (1983) demonstrated for Arctic charr in Norway that progeny from intramorph crosses of three coexisting morphs, differing in growth rates, produced all three adult forms. Laboratory rearing experiments with the progeny of the four morphs from Thingvallavatn also showed genetic component to differences in growth, age at first maturity, trophic morphology, body colour and foraging behaviour (Skulason et al. 1996).

Alexander and Adams (2004) placed offspring from individuals from four Scottish populations with differing adult morphologies under identical rearing conditions, including diet, and analysed measurements of head and jaw structure, at first feeding and at two and five months after first feeding. The results showed that the variation in head morphology reduced as the fish aged, allowing the conclusion that initial

variation is inherited as it is present prior to first feeding and therefore has a genetic component. However, with time, the magnitude of variation between the populations is reduced as a result of environmentally induced plasticity in phenotypic expression. This study is supported by that of Adams and Huntingford (2004), where different rearing environments were found to cause variation in head morphology of offspring from two morphs from Loch Rannoch, Scotland. Evidence from laboratory rearing experiments shows that morphs respond differently to variable environmental conditions i.e. the influence of phenotypic plasticity varies (Mikheev et al. 1996, Skulason et al. 1999). For example, small benthivorous charr from Thingvallavatn are less plastic than the planktivorous morph. This is not surprising as the availability of resources in the benthic niche is much more stable than in the pelagic (Skulason et al. 1999).

These studies have led to the conclusion that the development of morphs is partly influenced by both genetics and phenotypic plasticity. The relative importance of the two differs within and among systems and is likely to be dependent on past and present selective environments as well as developmental constraints such as the cost of plasticity. Where traits are of strong functional significance, the existence of different forms creates circumstances in which subsequent genetic divergence is favoured (Adams and Huntingford 2004). Therefore, the age and stability of the environment may well play a role in the relative contributions of phenotypic plasticity and the genetic component.

3.1.2.2. Ontogenetic differences in morphology

Arctic charr usually begin life as benthic feeders and therefore have morphological adaptations to benthic habitats. Some benthic morphs have matured at this ontogenetic stage, retaining their juvenile characteristics (Hindar and Jonsson 1982a, Sandlund et al. 1992). In these individuals, denoted 'dwarf' charr, growth will cease and they will remain small (Jonsson and Jonsson 2001). In other cases ontogenetic niche shifts may occur. As the immature fish grow, a proportion may move to the limnetic zone to feed on pelagic prey, and as a result they develop a more streamlined body form (Hindar and Jonsson 1982b). This usually occurs when the

fish is between 10 and 20 cm in length. Charr larger than 20-25 cm may switch to piscivory and the probability of this occurring increases with increasing body size (Amundsen 1995). The piscivorous morph then develops a more robust skull and jaw morphology (Fraser et al. 1998). Reasons for ontogenetic niche shifts are unknown but are likely to be density-dependent and morph transformation may be initiated by a reduction in surplus energy after reduced feeding opportunities (Forseth et al. 1994).

3.1.3. Arctic charr populations in the Lake District

The Arctic charr population in Windermere has traditionally been divided into two spawning populations, one that spawns in the autumn and one in spring (Frost 1965). The autumn spawning population spawn from October to December in shallow (1-4m deep) on the lakeshore and in the River Brathay, although the latter population may now be extint. Spring spawners, spawn from February to March, in water 15-20m deep (Frost 1965). These differences prompted research into the possible phenotypic differences between these two populations (Frost 1965, Partington and Mills 1988, Mills and Hurley 1990). The two spawning populations were found to differ in growth rate, with spring spawning fish having a faster growth rate than autumn (Partington and Mills 1988). The main morphological difference found between the two populations was that of gill raker number and gill raker length. Gill rakers are cartilaginous protuberances on the gill arches and are thought to be involved in the movement of water and food through the buccal cavity (Bone et al. 1999). Studies of Arctic charr feeding behaviour have found that limnetic morphs often have longer, finer gill rakers with narrower spacing between them, thought to increase the retention of zooplankton during filter feeding (Malmquist 1992). Partington and Mills (1988) found that spring-spawners in Windermere had a mean gill raker number of 14.7, and their gill rakers were long and thin, whereas, the autumn-spawners had a mean gill raker number of 12.9, and rakers were shorter and more robust. Variation between the populations was high so that this character could be used to accurately discriminate between individuals from the populations. Despite this, a study into differences in diet between the two populations was very limited, with very few fish studied (Mills 1989). Although unable to assess stomach

contents of spring-spawning charr, an assessment of parasite burden indicated higher levels of infection with *Diphyllobothrium* spp. than autumn spawners (Mills 1989). These parasites are transmitted through the consumption of infected copepods and are therefore associated with zooplanktivores suggesting that the differences in gill rakers may be attributed to feeding adaptations. Frost (1965) previously observed similar differences in gill raker number between the two spawning populations in Windermere, where mean numbers were 15.1 (range 13-17) for spring spawners and 13.3 (range 11-16) for autumn spawners, and attributed the differences to variation in temperature during early development.

Differences in parasite burden and growth rate have also been found between the two basins of Windermere with charr from both spawning populations from the south basin growing faster than those from the north basin. This can be attributed to the south basin being comparably more productive than the north basin (Mills 1989). These differences, as well as significant genetic differences (see Chapter 4.1) between basins for each spawning population, have led to the conclusion that movement between the basins is limited and that each basin holds separate stocks of each spawning population. Le Cren and Kipling (1963) used mark and recapture methods to estimate that in the early 1950s a combined total of 11000 mature charr spawned at three of the nine known autumn spawning sites whereas only 1950 charr spawned at one of the four known spring spawning sites, and therefore concluded that autumn spawners are far more abundant than spring spawners.

Coniston Water, a glacially excavated ribbon lake, is the third largest in England. It has an area of 4.9 km^2, maximum and mean depths of 56 and 24.1 m respectively (Partington and Mills 1988). The Arctic charr population of Coniston Water has been relatively little studied. However, some limited information is available. Unlike charr in Windermere, those in Coniston Water are reported to spawn only in the spring, in early March, despite local opinion that populations spawned in autumn and spring (Frost 1965). Partington and Mills (1988) assessed the morphological variation of Arctic charr populations from ten British lakes. The results showed that charr from Coniston Water had a mean gill raker length not significantly different

than that of the spring spawning charr in Windermere (Partington and Mills 1988) but were more morphologically similar to the autumn spawning population. Their diet also consists primarily of zooplankton species (Frost 1977). Wastwater lies within a glacially over-deepened valley behind a bedrock bar and is the deepest lake in the Lake District with a maximum depth of 79m. Wastwater was also included for study in Partington and Mills (1988) and individuals were found to overlap with those from Coniston Water and Windermere autumn spawners on the basis of morphology but gill raker length was not recorded for Wastwater.

3.1.4. Chapter aims

Morphological traits were measured in order to assess the range of phenotypic variation within the Arctic charr populations among lakes in the Lake District National Park, Cumbria, UK. For each lake, the objective was to investigate the pattern of morphotypic differentiation and to determine the relationship between morphology and feeding and spawning behaviour in order to test the following hypotheses:

1. The overall phenotypic variation of Arctic charr populations among the Cumbrian lakes will be high, as has been seen in other regions
2. Trophic interactions are important in the evolution of morphotypic differentiation of Arctic charr within the Cumbrian lakes
3. Spawning time and location will be correlated to morphotype within Windermere

3.2. Methods

3.2.1. Sample Collection

3.2.1.1. Collection from Coniston Water and Wast Water

Gill netting was undertaken by CEH Lancaster as part of a review and assessment of Arctic charr stocks in Coniston Water (Winfield et al 2005) and Wast Water (Winfield et al. 2006). Nets used were the standard version of the Norden survey gill net. The net, which is set on the lake bottom, is 1.5 m deep and 30 m long, with 12 panels of equal length of bar mesh sizes 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm. At Coniston Water, single nets were set at three offshore sites (north,

mid and south) on 15 June 2004 and a further four offshore sites (sites 1-4) on 16 June 2004, during the late afternoon or early evening (Table 3.1). Nets were then lifted the following morning and all fish were removed from nets and killed. The fish were then frozen at -20 °C within 6 hours to wait future processing in the laboratory.

At Wastwater, six benthic and three pelagic Norden survey gill nets were set singly for *c.* 24 hours at nine sites ranging in depth from *c.* 4 to *c.* 20 m on 10 August 2005. Sites 1, 4 and 7 were inshore with depth *c.* 4 m, while the remainder were all offshore with depth *c.* 20 m (Table 3.2). A total of 54 charr were caught from Coniston Water (Table 3.1); 26 males, 17 females and 15 immature fish, and a total of 19 fish were caught from Wastwater; 11 males, six female and two immature fish (Table 3.2).

Table 3.1. GPS locations for gill netting sites used at Coniston Water and the number of Arctic charr caught (taken from Winfield et al 2005). Locations are given in degrees and decimal minutes.

Netting site	Latitude	Longitude (West)	Number caught
North	54, 20.941	3, 4.115	0
Mid	54, 19.897	3, 4.860	1
South	54, 19.261	3, 5.035	9
Site 1	54, 19.202	3, 5.162	7
Site 2	54, 19.088	3, 5.256	15
Site 3	54, 18.926	3, 5.283	12
Site 4	54, 18.784	3, 5.301	11

Table 3.2. GPS locations for gill netting sites used at Wastwater and the number of Arctic charr caught (taken from Windfield et al. 2006). Locations are given in degrees and decimal minutes

Netting site	Latitude	Longitude (West)	Number caught
Site 1	54, 25.722	3, 19.049	0
Site 2	54, 25.660	3, 18.804	3
Site 3	54, 25.673	3, 18.740	0
Site 4	54, 26.777	3, 17.724	1
Site 5	54, 26.739	3, 17.739	7
Site 6	54, 26.746	3, 17.722	1
Site 7	54, 27.271	3, 16.219	0
Site 8	54, 27.214	3, 16.319	7
Site 9	54, 27.206	3, 16.410	0

3.2.1.2. Windermere sample collection

Gill netting of Windermere spawning fish was undertaken by CEH Lancaster as part of a long-term population study. The samples here were caught during the spawning periods, in the months of November, December, February and March between 2004 and 2006. The nets used were multifilament nets, 1.5 m deep and 30 m long with a mesh of 33 mm. Single nets were set on the lake bottom at known spawning sites in the early evening and lifted the following morning. Fish were removed from the nets, killed and frozen at –20 °C within 6 hours to wait future processing. Spring-spawning sites sampled were Holbeck and Rough Holme (north basin) and Rawlinsons Nab (south basin) and autumn spawning sites sampled were High Wray Bay, Rough Holme and North Thompson Holme (north basin) and Grass Holme and Sewage works (south basin) (Figure 3.1). Fish caught alive at North Thompson Holme were fin-clipped for genetic analysis (Chapter Four) and returned to the lake. The numbers of fish sampled from each site are shown in Table 3.3.

Table 3.3. Summarised information on the numbers of fish caught from each spawning site in Windermere

Site Name	Basin	Spawning Period	Sex		Total	
			Male	Female		
Rawlinsons Nab	South	Spring	7	21	28	
Holbeck	North	Spring	19	11	43	71
Rough Holme	North	Spring	7	6		
Grass Holme	South	Autumn	9	9	31	
Sewage Works	South	Autumn	6	7		
High Wray Bay	North	Autumn	6	2		56
North Thompson Holme	North	Autumn	11	0	25	
Rough Holme	North	Autumn	4	2		

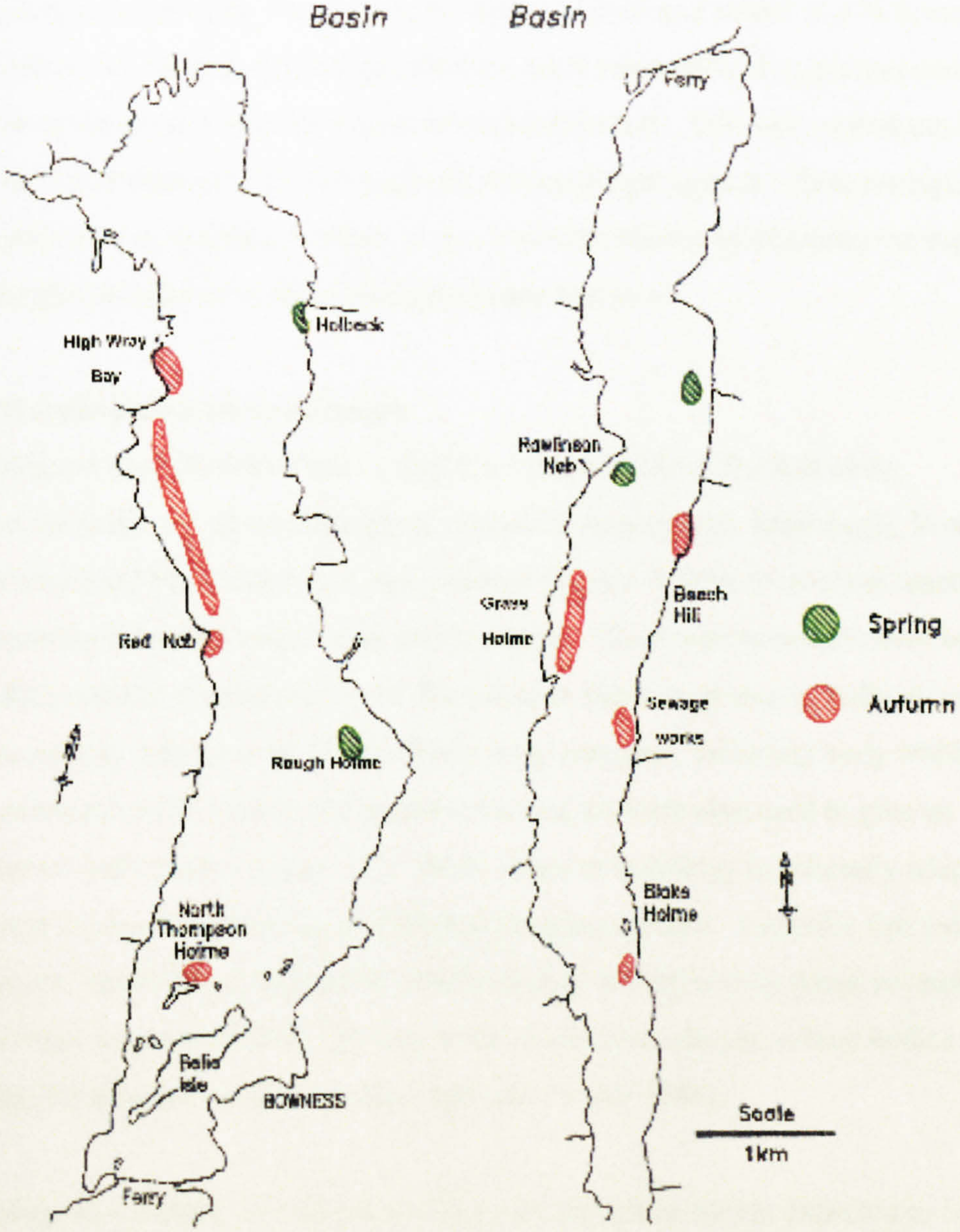


Figure 3.1. The historic distribution of autumn and spring spawning grounds of Arctic charr in Windermere, UK, adapted from Frost (1965)

3.2.2. Biological processing

After being partially thawed from storage at -20 °C, all fish were weighed, sexed and assessed as to maturity and spawning status if appropriate. The first gill arches of both the left and right gills were removed using a scalpel and stored in 4 % formalin for assessment of meristic characters. Otoliths were removed and examined under a binocular microscope to determine age of each individual. This was carried out by staff at the CEH Lancaster by viewing with reflected light against a dark background whilst immersed in methyl salicylate. Ages were determined by counting the bands visible on the otolith where each band represents one year.

3.2.3. Morphometric measurements

Morphological measurements were taken from the left side of the fish using precision callipers. Head measurements, including head length, head depth, lower jaw and maxillary bone length and eye diameter (Figure 3.3) were selected based on their importance for prey acquisition and handling. These measurements have been successfully used in several studies to discriminate between phenotypically divergent populations (e.g. Adams et al. 1998). Body measurements, including body width, caudle peduncle width, pelvic and pectoral fin lengths were also used to give an indication of body shape (Figure 3.2). Body shape morphology is generally adapted to different modes of swimming in different foraging habitats. Limnetic fish tend to have narrow, streamlined bodies and smaller fins to minimise drag when swimming in open-water whereas benthic fish tend to have relatively deeper, robust bodies and larger fins for manoeuvring in complex habitats (Webb 1984).

Morphological variation amongst individuals for any given trait is expected to be small relative to the size of the trait so can therefore be confounded by further variance such as measurement error. For this reason, care was taken to control for measurement error. All of the samples were measured by the author, using precision callipers. Each trait was measured to the nearest 0.01 mm and the callipers were reset to zero after every reading. Measurements were taken from the left side of the fish. If this was impossible due to damage, measurements from the right side were

taken. No measurements were attempted if structures were damaged on both sides of the fish, causing there to be some gaps in the data set. The traits measured were (Figure 3.2 and 3.3):

1. **FL** - Fork Length; distance from the tip of the snout to the fork of the tail fin.
2. **TL** - Total Length; distance from tip of the snout to the tip of the tail
3. **SL** – Standard length; distance from the tip of the snout to the end of the caudle peduncle
4. **CP** – Greatest depth of the caudal peduncle
5. **HL** – Head length. Distance from the tip of the snout to the operculum
6. **HDO** – Head depth at the operculum
7. **HDE** – Head depth at the eye
8. **LJ** – Length of the lower jaw
9. **MB** – Length of the maxilliary bone
10. **ED –** Diameter of the eye
11. **BD** – depth of the body at widest point
12. **PEC** – Pectoral fin length
13. **PEL** – Pelvic fin length

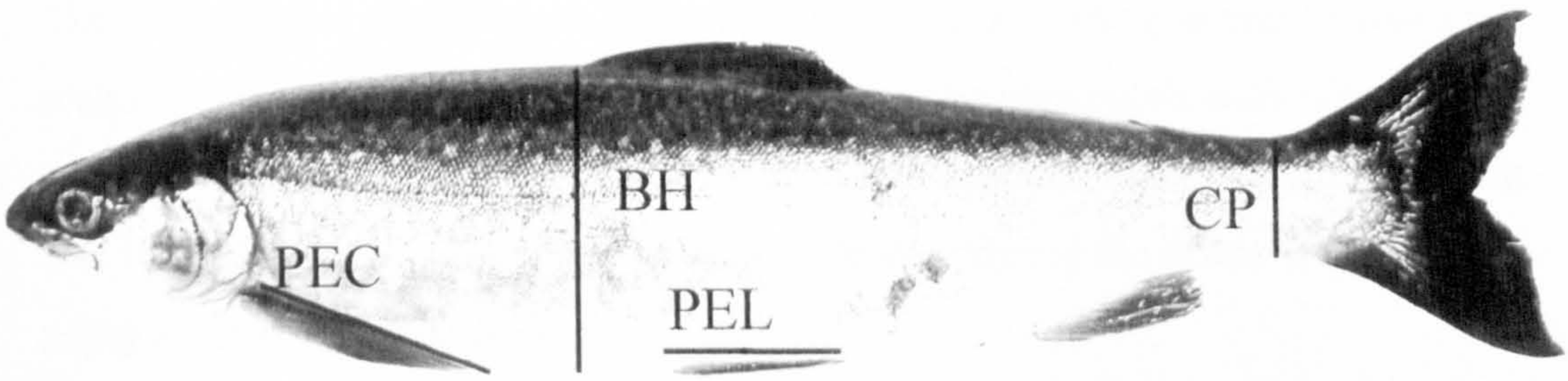


Figure 3.2. Body measurements used in the morphological analysis. BH, body width; CP, caudle peduncle width; PEC, pectoral fin length; PEL, pelvic fin length

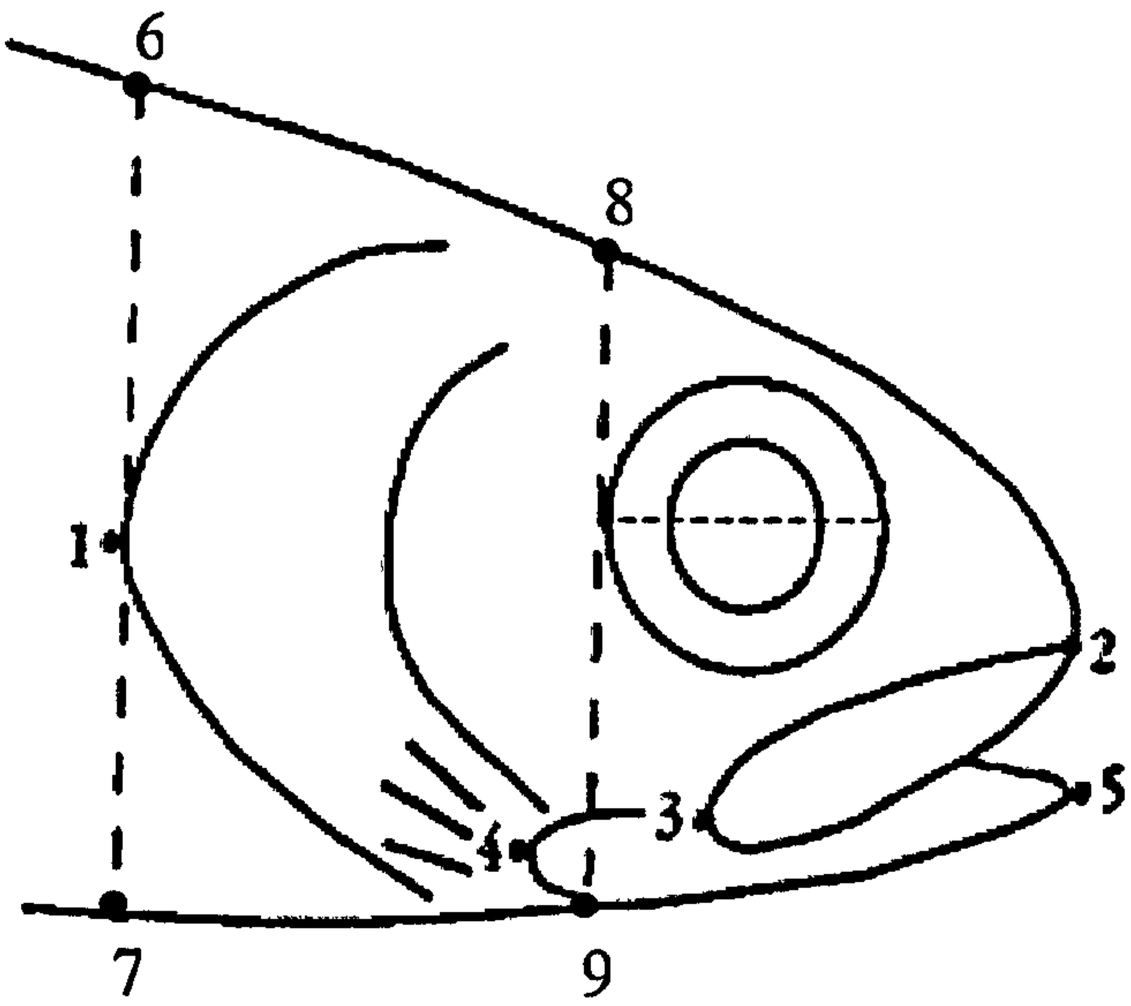


Figure 3.3. Head measurements used in the morphological analysis. HL, 1-2; HDO, 6-7; HDE, 8-9; LJ, 4-5; MB, 2-3. Adapted from Adams (1998).

3.2.3.1. Meristic characters

The total number of gill rakers was counted using a dissecting microscope at x5 magnification. Gill rakers on the lower arch and the upper arch were recorded separately. The raker at the apex of the arch was included in the lower arch count. The longest gill raker, usually situated next to the raker at the apex, was measured using a 1 mm eyepiece graticule.

3.2.4. Dietary analysis

Details of methods of dietary analysis used and information on the diet of Windermere populations discussed here are given in Chapter Two. The results of dietary analysis of Coniston Water populations are given here. Details of the methods and statistical analysis are given in Chapter Two. The stable isotope analysis of white muscle tissue could not be performed on charr from Coniston Water, as none was available, and instead, scales were used (Syvaranta et al. 2008).

The analysis was carried out by C. Harrod, Queen's University, Belfast. The scales were treated with hydrochloric acid to remove inorganic carbonate and oven dried at 60 °C for 24 hours. The dried scales were then homogenised using an agate pestle and mortar and stored in a desiccator. The stable isotope ratios were then determined using continuous flow isotope ratio mass spectrometry as described in Chapter Two.

3.2.5. Statistical analysis

Most of the variability in a set of multivariate morphometric data from a natural population is due to the variability in individual size. To remove the influence of size prior to analysis, all fish from all populations were pooled before the data was $\log_e$-$\log_e$ transformed to homogenise variance prior to analysis (Adams et al. 2003). For each population a multivariate analysis of variance (MANOVA) was used to test for differences in the morphological characters between sexes. Principal component analysis (PCA) was used to characterise morphology and explore variation of fish morphology among lakes. The significance of separation of lakes on the basis of population morphology was tested using pairwise one-way analysis of variance (ANOVA).

Within lake phenotypic variation was also examined in both Coniston Water and Windermere populations, using PCA. The separation of individuals on the basis of morphology is determined by weighting all the available morphological characters to provide the maximum discrimination between individuals. In Windermere comparisons of principal component scores of fish divided into their known putative spawning populations (see section 3.1.3) was made in the same way as between lakes, using one-way ANOVA. Comparisons of size and age structure between spawning populations was also made using one-way ANOVA.

3.3. Results

3.3.1 Phenotypic variation among lakes in the Lake District

The principal component (PC) one coefficients showed large positive loadings for eye diameter, head depth at the eye and operculum against large negative loadings for head length and lower jaw length (Table 3.4). This indicated that charr with

relatively robust head shapes and large eye diameters for a given size would score highly at PC1. PC2 showed large positive loadings for all traits linked to body shape, body width and caudal peduncle width and fin lengths. This indicated that charr with relatively deep bodies and long fin lengths for a given size would score highly at PC2. Large standard deviations for both PC1 and 2 for Coniston Water and at PC2 for Windermere indicated that within lake phenotypic variability was also high. There was significant variation in morphology across the lakes examined. Amongst the three populations examined here there was significant between-population variation in both PC1 scores ($F_{1,3} = 111.1$; $P < 0.001$) and in PC2 scores ($F_{1,3} = 25.5$; $P < 0.001$) (Figure 3.4). Pairwise analysis of component scores for both PC1 and PC2 showed significant differences between two out of three lake pairs (67 %) (Table 3.5).

Table 3.4. Component loadings from PCA performed on transformed morphometric data from Arctic charr from Windermere, Coniston Water and Wast Water. The first two principal components account for 80.6 % of the variation.

Variable	Component	
	1	2
EYE	.912	.178
HDE	.870	.376
HDO	.665	.586
BH	.138	.672
PEL	-.363	.707
CP	-.423	.681
PEC	-.448	.634
LJ	-.724	.516
HL	-.948	.134
Eigenvalue	**4.68**	**3.38**
Cumulative % Variance	**46.8**	**80.6**

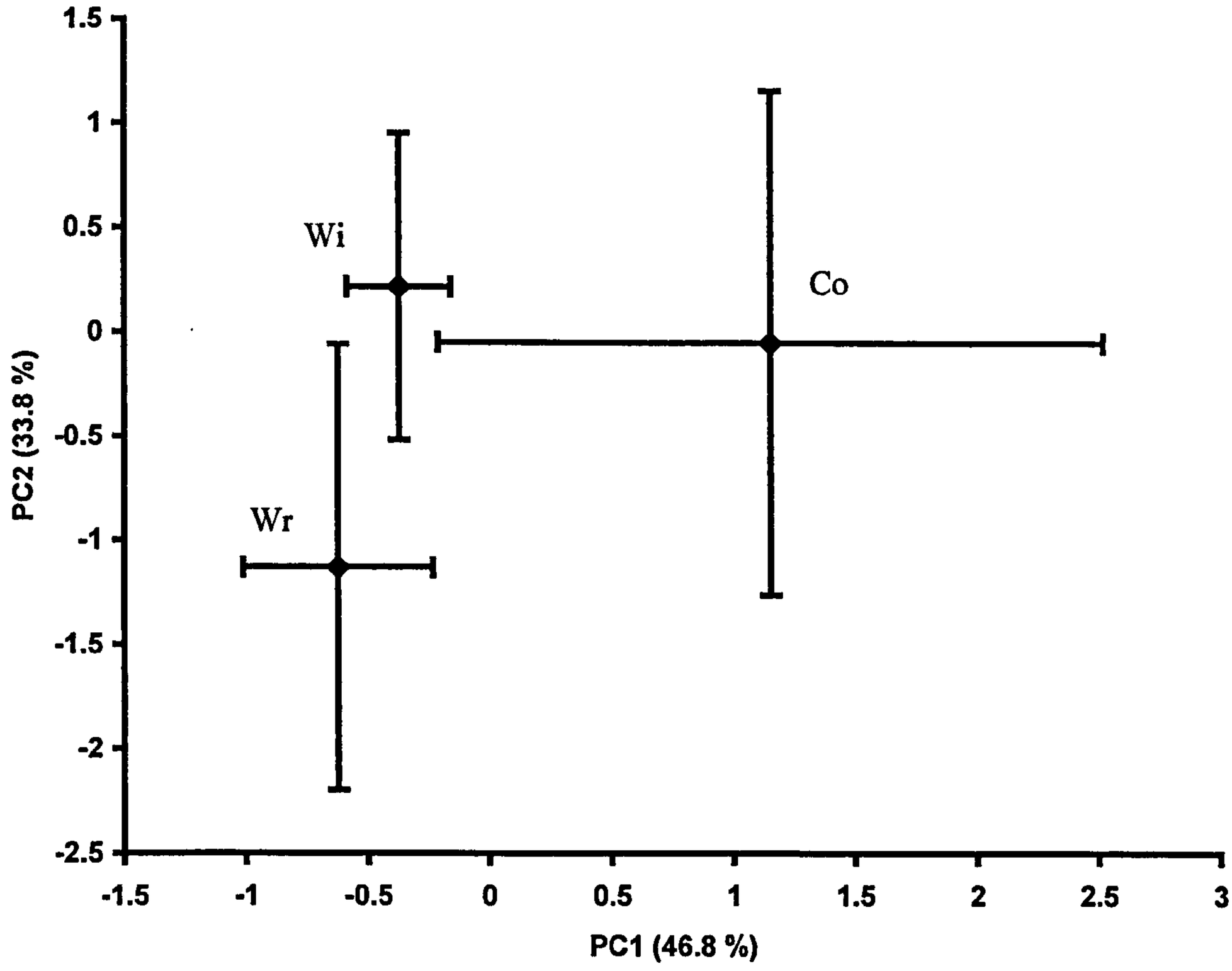


Figure 3.4. Lake mean and standard deviation of principal component 1 (PC1) versus PC2 scores of morphology of Arctic charr. Wi, Windermere; Co, Coniston Water; Wr, Wast Water.

Table 3.5. Pairwise lake comparisons (Bonferroni correction applied) of PC1 (Below the diagonal) and PC2 (above the diagonal) scores. Wi, Windermere; Co, Coniston Water; Wr, Wast Water. * < 0.05, ** <0.001

	Wi	Co	Wr
Wi		**NS**	**
Co	**		**
Wr	**NS**	**	

3.3.2. Phenotypic variability within lakes

3.3.2.1. Windermere

Significant differences in morphology were found between the sexes in the Windermere spawning sample (MANOVA $P < 0.001$). Additional analysis of the morphology of those fish caught in late summer (September) (Chapter Two) also found significant differences between the sexes (MANOVA; $P < 0.05$). Univariate analysis showed the significant variables are those related to head shape, including head length, depth at the eye, and lower jaw length as well as pelvic fin length. Further analysis was therefore carried out on each sex separately. Each sample contained fish at all ages between 3 years and 7 years and both showed similar patterns of mean length with age (Figure 3.5) but the females' attained significantly greater lengths in the first two age classes (ANOVA; $P < 0.01$). Ontogenetic niche shifts are thought to occur when fish reach 100–200 mm; therefore no fish below 200 mm were included in the analysis.

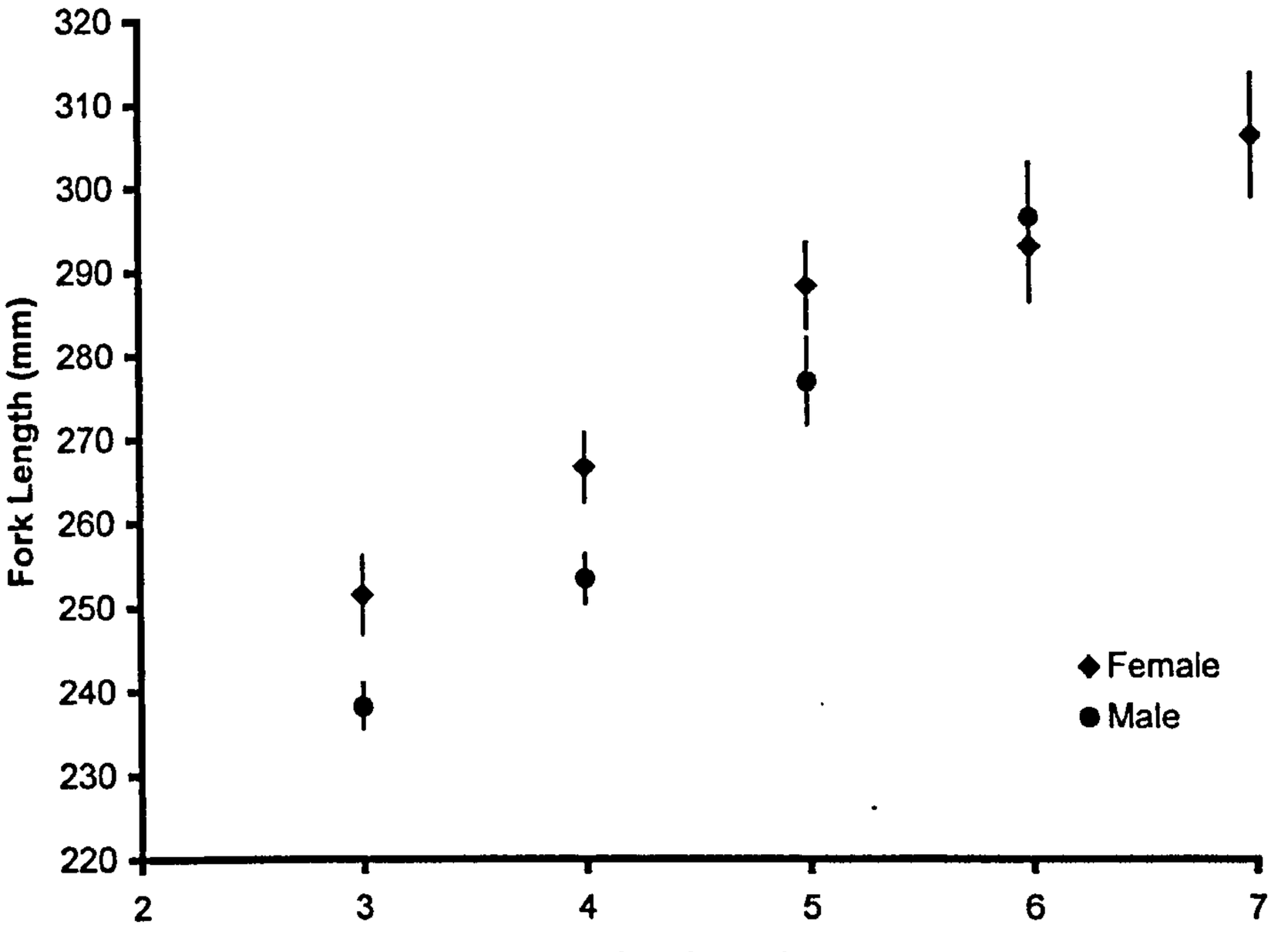


Figure 3.5. Mean fork length (± S.E.) at age of male and female charr from Windermere using samples combined from both basins (Table 3.3.).

The numbers of fish successfully aged were too low to test for differences in size and age structure between spawning population in each basin individually therefore they were combined. Table 3.6 gives the mean fork length (±1 s.e.) at each age class for autumn- and spring-spawning individuals. The mean fork length was not significantly different between autumn- and spring-spawning sub-populations in any age class (ANOVA).

Table 3.6. Mean fork length (± 1 s.e.) for each age class of male and female charr from autumn- and spring-spawning sub-populations in Windermere, using sample combined from both basins. The number of individuals aged is given in parentheses.

Sex	Spawning season	Age Class				
		3	4	5	6	7
Male	Autumn	237±3.8 (3)	251±4.7 (5)	276±8.3 (4)	303 (1)	-
	Spring	241 (1)	257±6.1 (15)	279±6.9 (3)	-	284±4.9 (2)
Female	Autumn	-	259±5.8(13)	288±5.6 (10)	300±9.7 (4)	301±4.9 (2)
	Spring	-	263±3.8 (4)	291±7.4 (7)	268 (1)	312±6.5 (2)

The Principal Component Analysis (PCA) of size corrected-morphometric variables showed that the first three components accounted for 60.12 % of the morphological variation in males and 60.38 % of the variation in females. In males the PC1 components were all positive and those for head shape were highly positive. This indicated that those fish with high PC1 scores would have large, robust heads and deeper bodies. PC2 showed large, positive component loadings for pelvic and pectoral fins against negative loadings for body height, head depth and caudle peduncle width. This suggested that male fish with relatively long pectoral and pelvic fins and shallow body shape would score highly at PC2 (Table 3.7).

In females, PC1 component loadings were all positive and again those for head shape were highly positive, suggesting fish with high PC1 scores will also have relatively large robust heads. PC2 showed large negative component loadings for eye

diameter against positive loadings for body height therefore fish with relatively smaller eyes and deeper bodies would have high PC2 scores (Table 3.8). There was significant variation in morphology within the Windermere spawning populations, indicated by significant differences in both PC1 scores ($F_{3,62} = 4.7$; $P < 0.01$) and PC2 scores ($F_{3,62} = 12.1$; $P < 0.001$) in males (Figure 3.6) and significant differences in PC2 scores ($F_{3,45} = 4.2$; $P < 0.01$) in females (Figure 3.7). In males the pairwise comparisons of component scores showed differences between the autumn-spawning south basin population and all the other populations for PC1 and between the autumn and spring-spawning populations in the south basin and between the autumn-spawning population in the south basin and spring-spawning population in the north basin for PC2 (Table 3.9). In females, pairwise comparisons showed significant differences between the mean PC2 scores of spring and autumn-spawning populations in the south basin only (ANOVA; $P = 0.004$). No significant differences were found for PC1.

There was no significant difference in gill raker number or gill raker length between the sexes in Windermere. Therefore the samples were pooled for further analysis. The north basin spring group had a significantly higher mean number of gill rakers than the other spawning groups (Table 3.12) (ANOVA: $P < 0.01$). There was, however, no significant difference in the mean gill raker length between the putative spawning populations of Windermere.

Table 3.7. Component loadings from PCA performed on transformed morphometric data from male Arctic charr from Windermere. The first three principal components account for 60.12 % of the variation.

Variable	Component		
	1	2	3
HDO	**.806**	-.351	.029
BH	**.785**	-.184	.257
HL	**.777**	.188	-.233
HDE	**.668**	-.151	-.505
LJ	**.636**	.100	.433
CP	.620	-.351	.055
Weight	.484	.277	.076
PEL	.314	**.792**	-.067
PEC	.219	**.637**	-.271
Gill raker length	.079	.385	**.739**
EYE	-.030	.544	-.001
Eigen value	**3.490**	**1.903**	**1.220**
Cumulative % Variance	**31.725**	**49.030**	**60.121**

Table 3.8. Component loadings from PCA performed on transformed morphometric data from female Arctic charr from Windermere. The first three principal components account for 60.38 % for females.

Variable	Component		
	1	2	3
CP	**.806**	-.051	-.105
HL	**.792**	-.095	.246
LJ	**.779**	.043	.018
HDO	**.679**	.554	-.025
PEC	**.679**	-.376	.318
HDE	.668	-.247	.075
PEL	.645	-.312	.143
Weight	.449	**.330**	-.313
BH	.426	**.700**	-.372
EYE	.361	**-.715**	-.515
Gill raker length	.211	.425	**.601**
Eigen value	**4.289**	**1.715**	**1.242**
Cumulative % Variance	**35.739**	**50.029**	**60.381**

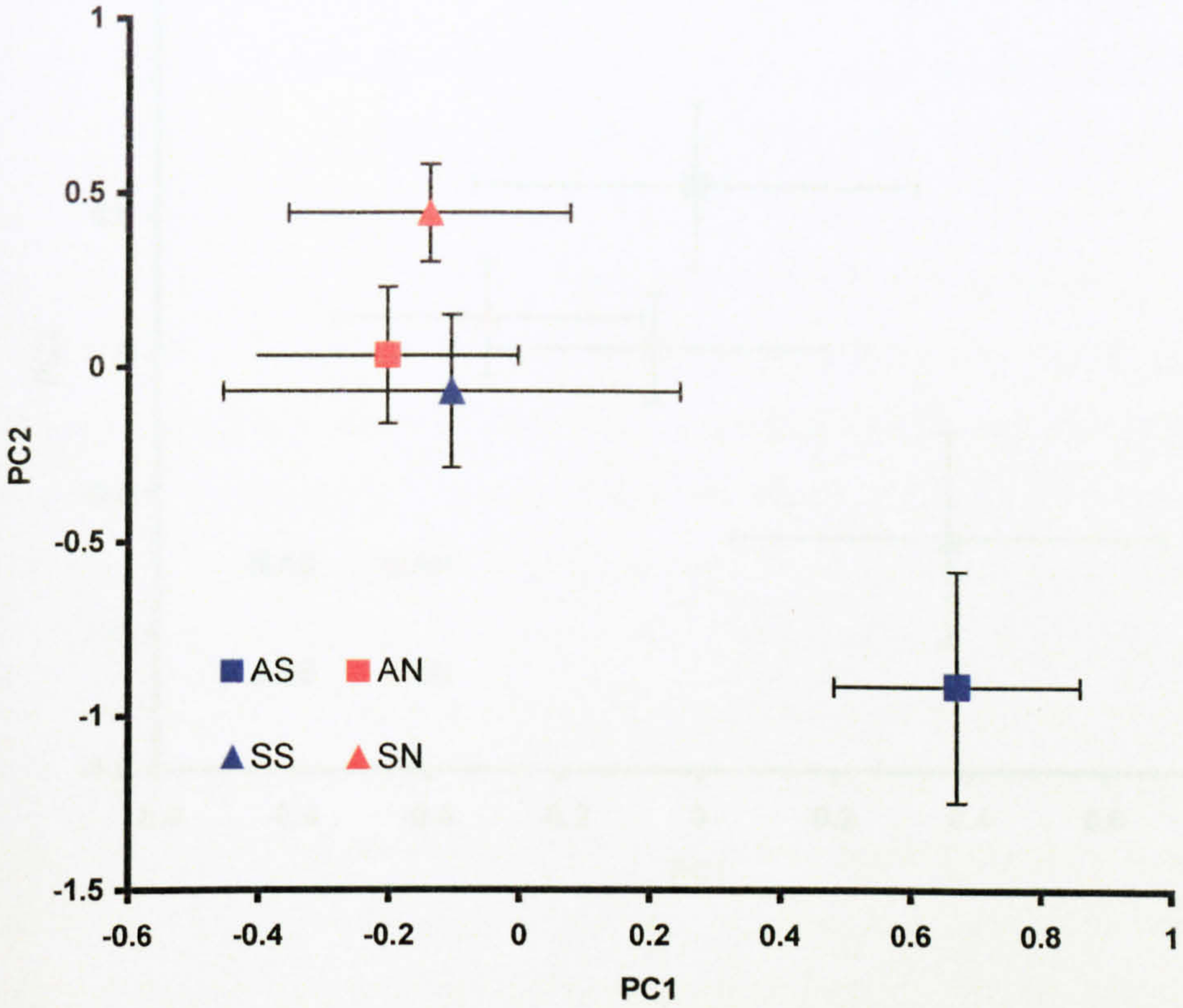


Figure 3.6. Mean Principal component scores (± S.E.) at PC1 and PC2 for male Arctic charr from spawning populations in Windermere (AS, autumn south; AN, autumn north; SS, spring south; SN, spring north).

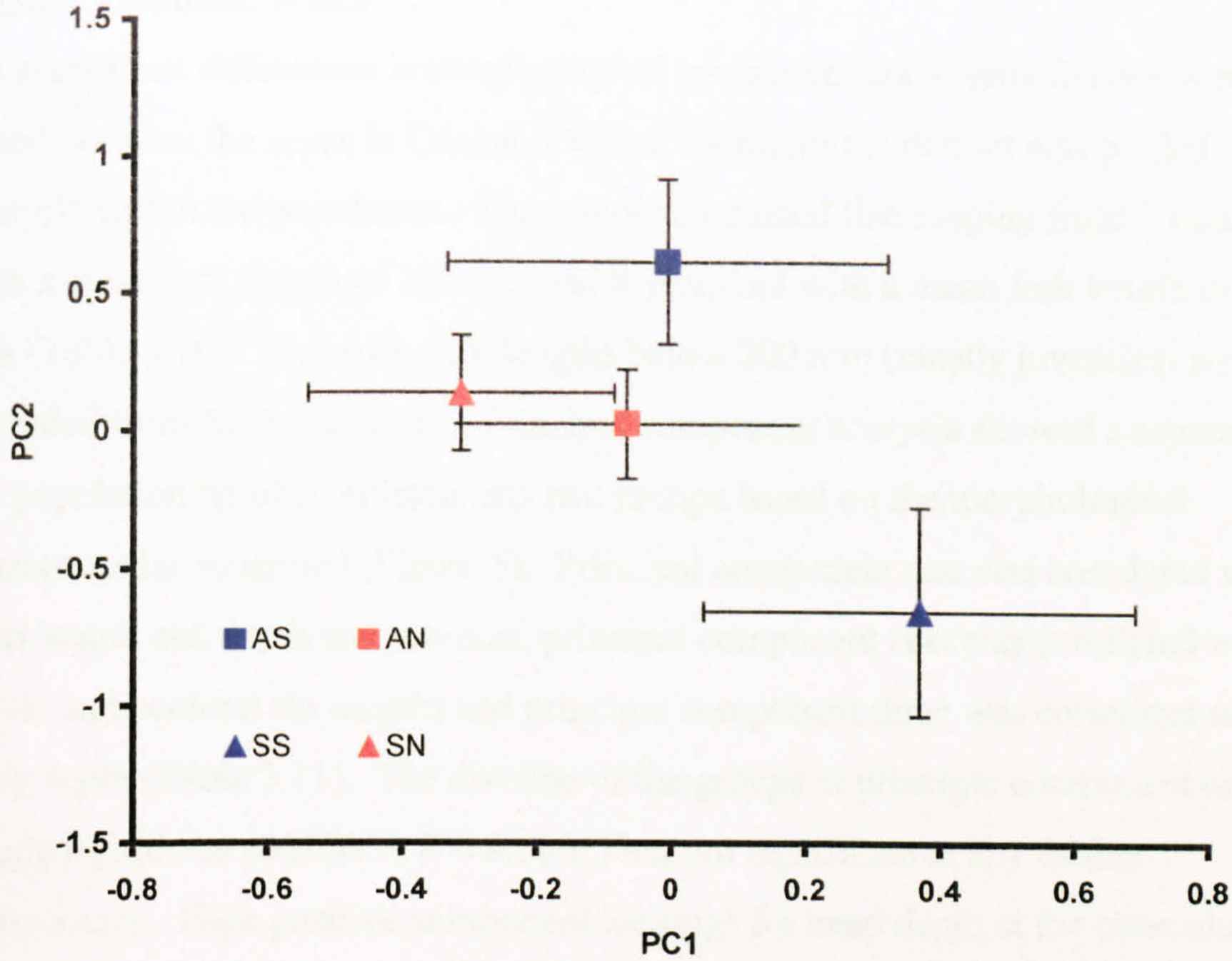


Figure 3.7. Mean Principal component scores(± S.E.) at PC1 and PC2 for female Arctic charr from spawning populations in Windermere (AS, autumn spring; AN, autumn north; SS, spring south; SN, spring north).

Table 3.9. Results of Bonferroni Posthoc tests of ANOVA of PC1 (below diagonal) and PC2 (above diagonal) scores between pairs of spawning populations of male Arctic charr in Windermere. AS, autumn south; AN, autumn north; SS, spring south; SN, spring north; ** significant at 0.01

	AS	AN	SS	SN
AS		**	NS	**
AN	**		NS	NS
SS	**	NS		NS
SN	**	NS	NS	

3.3.2.2. Coniston Water

No significant differences in morphological measurements or growth rates were found between the sexes in Coniston Water; therefore the dataset was pooled for analysis within the population. The sample contained fish ranging from 2 years old with a mean fork length of 135 mm and 8 years old with a mean fork length of 317 mm (Table 3.10). Fish with fork lengths below 200 mm (mostly juveniles) were excluded from further analysis. Principal component analysis showed a separation of the population within Coniston into two groups based on the morphological characteristics measured (Figure 5). Principal component one was correlated with head length and depth and jaw size, principal component two was correlated with pelvic and pectoral fin lengths and principal component three was correlated with body depth (Table 3.11). The division of the groups at principle component one was highly significant (ANOVA $P < 0.0001$) but not significant at any further components. High positive component loadings for head depth at the operculum (HDO) and maxillary bone length (MB) and high negative component loadings for head length (HL) and lower jaw (Kiljunen et al.) (Table 3.11) suggest that those fish with deep heads and large maxillary bone lengths have lower head lengths and lower jaw lengths and vice versa. Therefore fish with positive PC1 scores are those with deep, short heads and large maxillary bone lengths. These individuals comprised approximately two thirds of the sample size. Those fish with negative PC1 values have long narrow heads and smaller lower jaws and comprise approximately one third of the sample (Figure 3.8).

Mean gill raker number and gill raker length of samples from Coniston Water divided into the two morphotypes defined by the PCA are given in Table 3.12. The delicate morph in Coniston Water had a slightly higher mean number of gill rakers on the first gill arch (23.53 for the delicate morphs *cf.* 22.12 for the robust morphs) than the robust morph but this was non significant (ANOVA).

Table 3.10. Mean fork lengths (mm) and numbers (in parantheses) of aged male and female Arctic charr from Coniston Water.

Sex	Age (years)						
	2	3	4	5	6	7	8
Male		155 (3)	207 (6)	216 (16)	279 (3)		
Female	135 (1)	171 (2)	223 (5)	230 (12)	271 (1)		317 (1)

Table 3.11. Component loadings from PCA on transformed morphometric data from Arctic charr from Coniston Water. The first three principal components account for 72.89 % of the variation

Variable	Component		
	1	2	3
HL	-.972	.027	.093
LJ	-.936	.095	.046
HDE	.934	.277	-.067
MB	.925	.280	-.172
ED	.870	.243	.047
HDO	.701	.285	.292
PEL	-.360	.768	-.405
CP	-.360	.353	.114
PEC	-.350	.724	-.523
BD	-.123	.335	.712
Snout bluntness index	-.122	.450	.351
Gill raker length	-.116	.527	.414
Eigen value	**5.223**	**2.136**	**1.385**
Cumulative % Variance	**43.525**	**61.328**	**72.869**

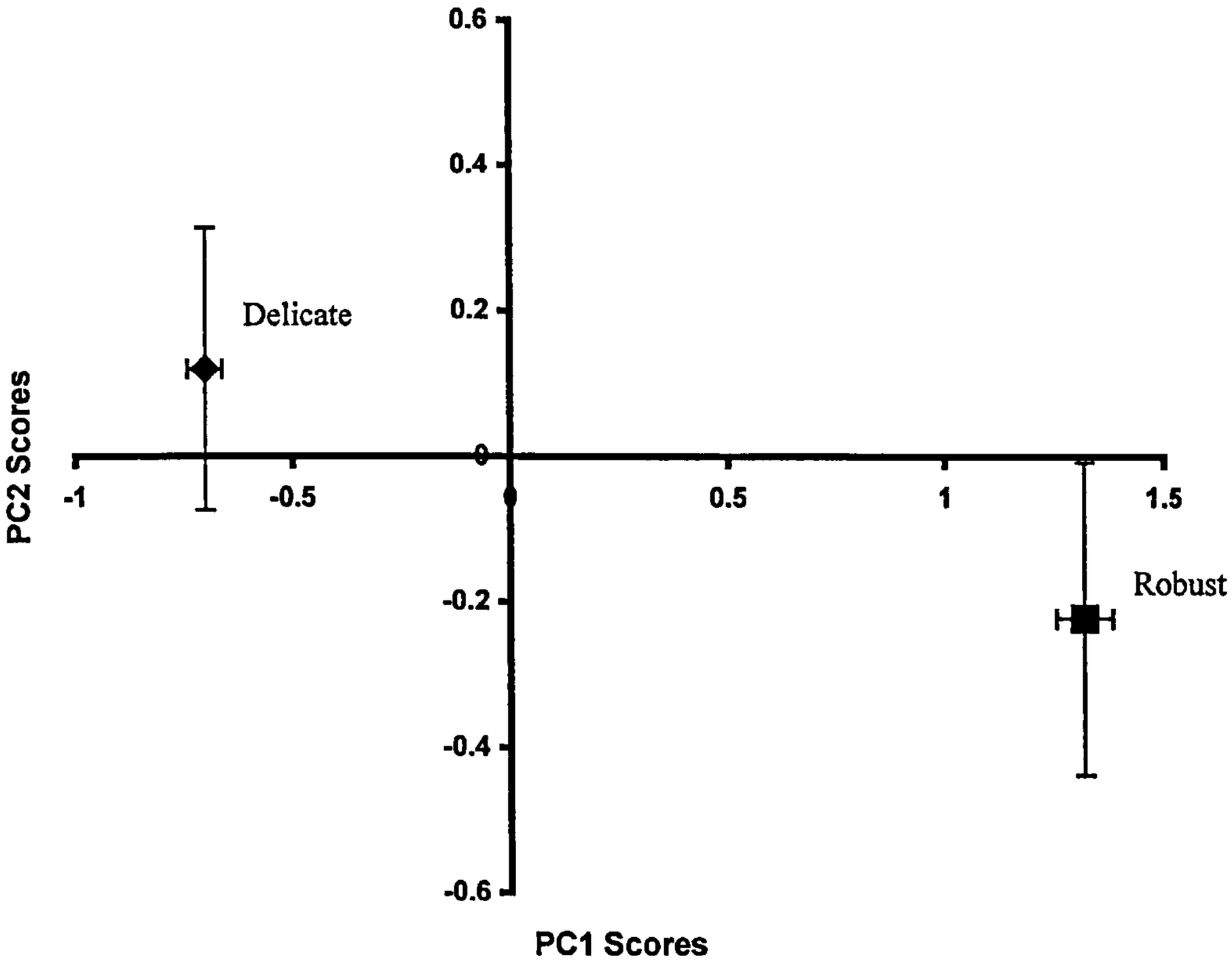


Figure 3.8. Mean and 1 s.e. Principal component scores at PC1 and PC2 for two morphs of Arctic charr in from Coniston Water.

Table 3.12. Comparison of gill raker numbers and maximum lengths for putative spawning populations in Windermere and morphs in Coniston Water

Population	Gill rakers on 1st gill arch		Gill raker length (mm)	
	Mean	S.E.	Mean	S.E.
Windermere:				
Autumn south	21.36	0.24	4.12	0.09
Autumn north	21.39	0.24	4.06	0.22
Spring south	21.15	0.28	3.88	0.12
Spring north	22.79	0.34	4.35	0.15
Coniston Water:				
Delicate morph	23.53	0.26	3.65	0.17
Robust morph	22.12	0.26	4.08	0.13

3.3.3.2. Dietary analysis of Coniston Water

Of the 54 fish caught from Coniston water seven had empty stomachs. The other fish had a range of prey items in their stomach contents including zooplankton (Cladocera), and benthic invertebrates, including, chironomids, isopods (*Asellus*) and amphipods (*Gammarus*). Cladocera was the predominant prey type, occurring in 85 % of fish, including those from both morphological groups. There was, however, some segregation between the two morphological groups (Figure 3.9). There were fewer fish containing zooplankton prey in the group with more robust heads and they also contained benthic invertebrates whereas fish with delicate head structure consumed zooplankton only.

The carbon and nitrogen stable isotope values of scales from the two sub-groups of Arctic charr from Coniston Water showed considerable overlap. The delicate morph had lower mean values of both carbon and nitrogen than the robust morph but only by 0.2 ‰ and 0.3 ‰ respectively and thus was not significantly different (Figure 3.10).

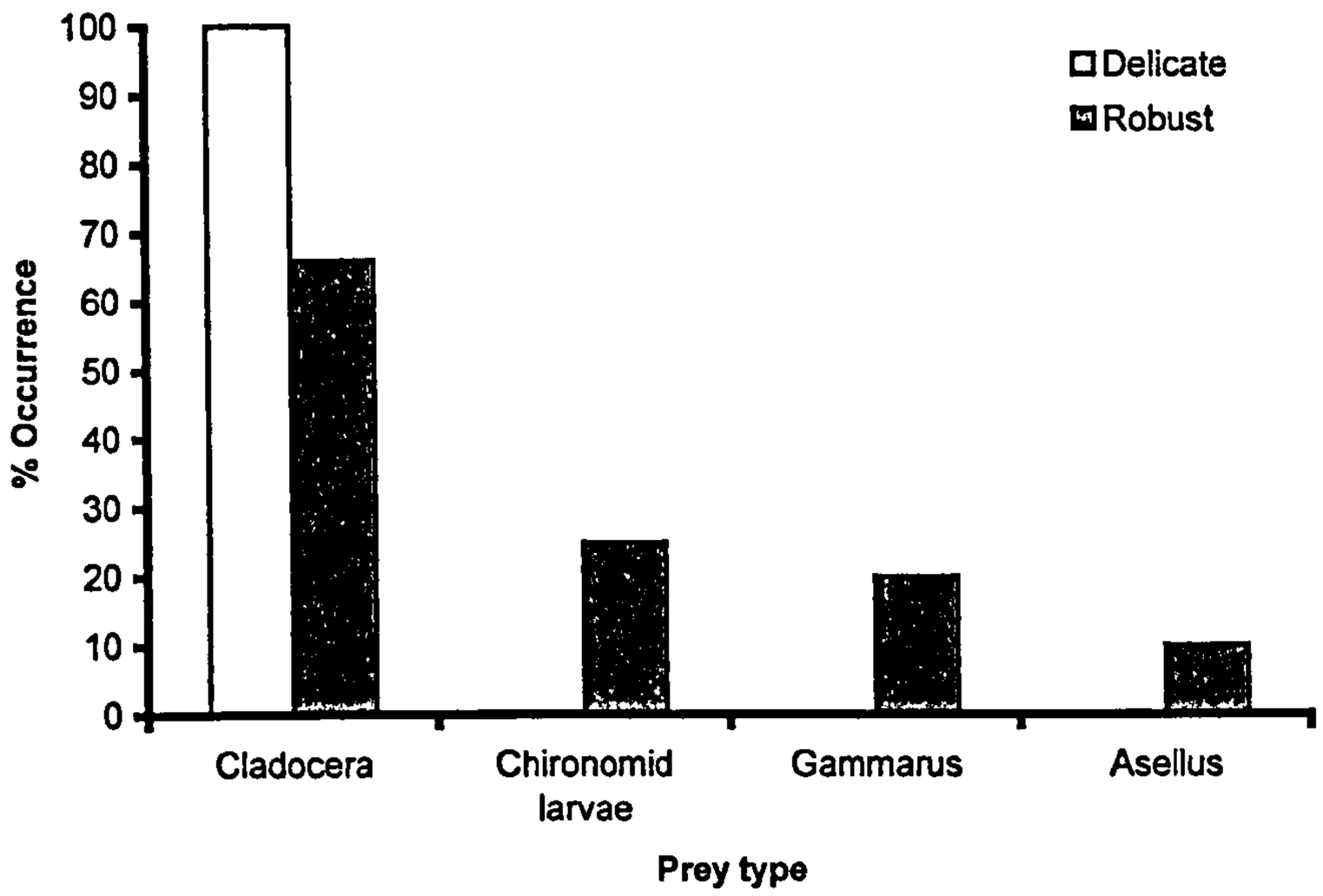


Figure 3.9. Percentage occurrence of each of the prey items in the diets of the two morphs from Coniston Water

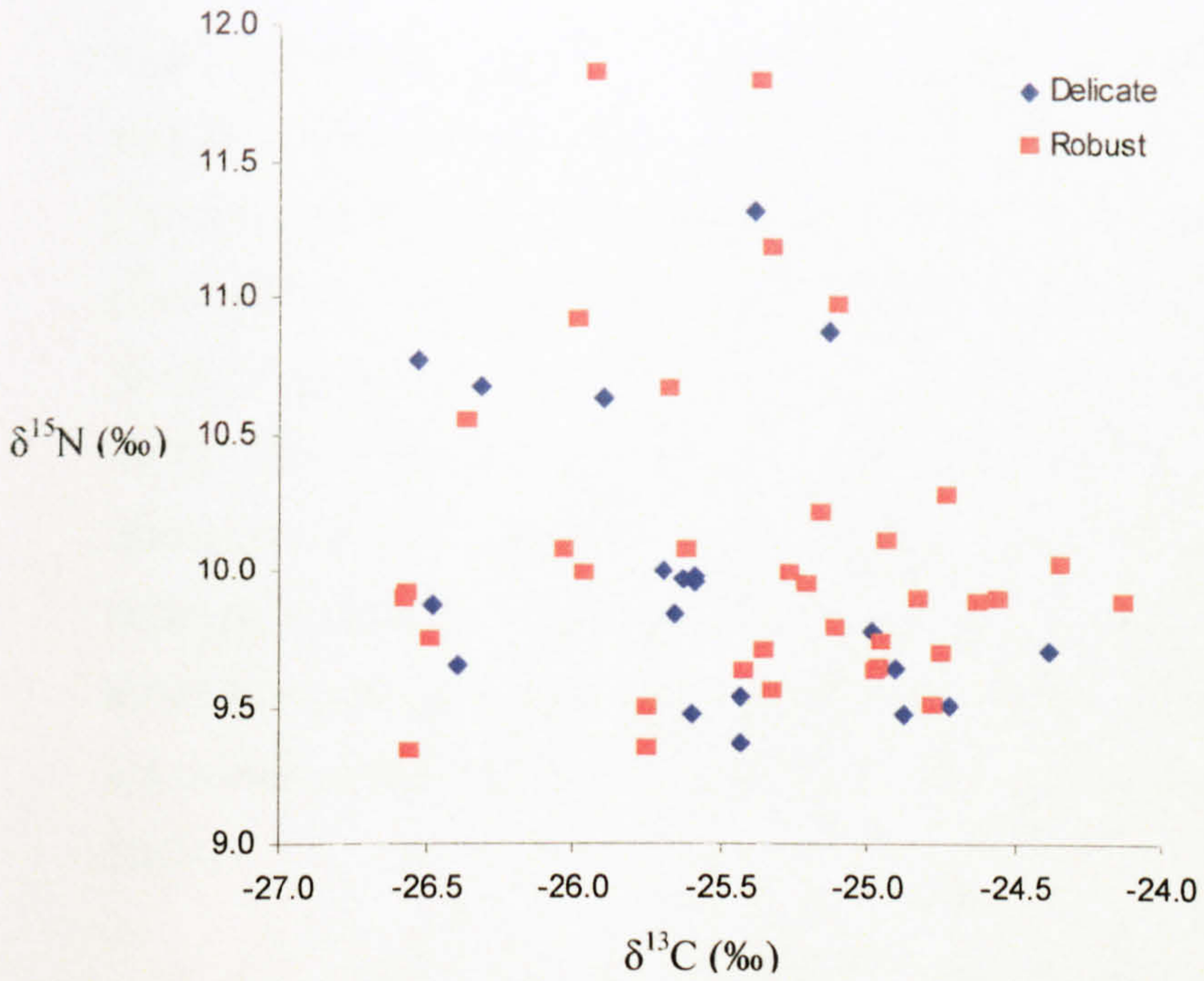


Figure 3.10. Mean $\delta^{13}C$ and $^{\delta 15}N$ values (± S.E.) of Arctic charr scales from morphs in Coniston Water

3.4. Discussion

3.4.1 Phenotypic variability among lakes in the Lake District

Recent analyses of Arctic charr populations throughout their range have revealed high levels of phenotypic variability and significant phenotypic differentiation between populations on small spatial scales. Adams et al. (2007) present evidence of significant phenotypic variation across the Arctic charr over the whole of Scotland, as well as significant differentiation of populations both among catchments and among lakes within catchments. This study provides evidence of variation in morphology over very short geographic distances. The maximum distance between these lakes is 30 km and all are thought to have been colonised by Arctic charr at similar times by one common ancestor (Wilson et al. 2004). This suggests that the phenotypic variability observed in the Lake District has arisen since the lakes' formation ~15,000 years ago, and reflects a similar level of variability to that observed amongst charr populations elsewhere

The morphological characteristics accounting for much of the variation amongst and within the lakes were head measurements, including head length, depth and lower jaw length, along with body depth and caudle peduncle width. These morphological characteristics have been shown to differ repeatedly between sympatric morphs of Arctic charr in many lakes (e.g. Snorrason et al. 1994, Reist et al. 1995, Adams et al. 1998). These characteristics have been shown to have clear functional significance, with lower jaw size being directly linked to the handling of prey items (Adams and Huntingford 2002a, Fraser et al. 2007) whereas body shape and fin size are associated with locomotion in distinct habitats (Webb 1984), not only in Arctic charr but in other fish species as well (e.g. Amundsen et al. 2004, Parsons and Robinson 2007).

3.4.2. Phenotypic variability within lakes in the Lake District

3.4.2.1. Windermere

As anticipated, the results of the present study have shown high levels of phenotypic variation within Windermere. The sexual dimorphism found was unexpected but perhaps not surprising given that the charr were caught during spawning periods and salmonids are well known for their development of secondary sexual characteristics. For example, in the Pacific salmon, genus *Oncorhynchus*, changes in males include the development of a hooked snout or 'kype', a dorsal hump, and changes in skin colouration. However, this is an extreme example and expression of secondary sexual characteristics is not so great in *Salvelinus* species (Fleming and Reynolds 2003). Despite this, observations of some such changes have been seen in Scottish Arctic charr (C. Adams, pers. comm.) but are not well documented. Changes in skin colouration in Arctic charr, however are very common, with males changing from a silvery colouration to bright red breeding colours (Balon 1984) during the spawning period. Despite this, significant differences between males and females were also found within the samples obtained in September, which suggests that either sexual dimorphism may be retained all year round or that these fish were already beginning to display spawning characteristics prior to the autumn spawning period.

When analysed separately, both sexes still show large amounts of phenotypic variability within Windermere. When separated by spawning group, however, there was little distinction between them based on morphology. In males, the south autumn spawning population was the only group significantly different from the other populations at PC1. This group had a much higher mean PC1 score, indicating the charr in this group had relatively large, robust head shapes (Figure 3.6), typical of feeding on benthic invertebrates (Adams and Huntingford 2002a, Andersson 2003, Andersson et al. 2005). In females the differentiation between the four spawning populations is less distinct with large overlap in PC1 scores between populations. There is some degree of separation at PC2 with significant differences between the spring and autumn spawning populations in the south basin. The spring spawning population from the south basin had a lower PC2 score suggesting shallower body morphology compared to the autumn spawning population (Figure 3.7). This 'shallow body' type of morphology is typical of feeding within the water column. Thus when analysed separately both sexes showed some variation in morphology but only in the south basin. Here both sexes showed significant differences between spawning groups but the variation is not consistent with the pelagic or benthic morphology described in the literature.

The number and length of gill rakers have been used to differentiate between autumn and spring-spawners in Windermere in the past (Frost 1965, Partington and Mills 1988, Kottelat and Freyhoff 2007). This study, however, found no significant differences in the number of gill rakers or in the length of the longest gill raker. It is possible that this is a plastic trait and these differences have been lost due to changes in diet. On the other hand, the counting of gill rakers is very subjective as there is no set protocol, and discrepancies between the past studies were also found. Partington and Mills (1988) consistently recorded lower gill raker counts than Frost (1965). Therefore, in absence of any knowledge on how past studies made their measurements, it is very difficult to make comparisons with the present study. There were also no differences in mean fork length with age between the spawning populations in Windermere, as has been found previously (Partington and Mills 1988).

Although the evidence from dietary analysis is limited (Chapter Two) and data from males and females had to be pooled due to low sample sizes, it is consistent with morphological findings to some degree. The autumn spawning south population had high $\delta^{13}C$ and $\delta^{15}N$ ratios (-24.3 ‰ and 14.6 ‰ respectively), which are significantly greater than the autumn and spring spawning populations in the north basin but not the spring spawning population in the south basin. As discussed in Chapter Two, this may reflect differences in baseline carbon and nitrogen values between the basins rather than true differences in diet. That aside, the isotopic signatures seen would suggest that the autumn spawning south population had a diet consisting of a larger proportion of benthic invertebrates, consistent with their benthic morphology, for at least part of the year. However, this pattern is not as pronounced as has been found in lakes elsewhere, where stable isotope analysis, parasitology or dietary analysis have revealed complete ecological segregation of morphotypes that is stable over time (Malmquist et al. 1992, Adams et al. 2003). The wider range of stable isotope values and in diet amongst spawning individuals (Chapter Two) may suggest a more generalist strategy amongst the charr in Windermere compared to elsewhere. This may be attributed to seasonality and the availability of different prey items in Windermere compared to lakes at higher altitudes or latitudes. Windermere is at the southern most point of the geographical range of Arctic charr at such a low altitude, in a temperate climate. Here, food availability of different prey items is likely to be less variable over the year (see discussion, Chapter Two) than in lakes at higher altitudes or latitudes.

3.4.2.2. Coniston Water

In Coniston Water the Arctic charr sampled separated into two discrete groups based on their morphology with little overlap. Again, head measurements including lower jaw length, head length and depth were most important in distinguishing between the two groups. These traits are those that have been found repeatedly in many sympatric populations where benthic and pelagic morphs are found (Adams et al. 1998, Adams et al. 2003, Andersson 2003, Blackie et al. 2003, Adams et al. 2007, Bush and Adams 2007). In the majority of examples these morphs are associated

with foraging in benthic and pelagic habitats and thus dietary studies have found their diet composition to reflect that of the habitat in which they forage (e.g. Adams et al 1998). In this case zooplankton species were the most abundant prey items in the diet of both morphs (Figure 3.9). However, the diet of the benthic morph did include benthic invertebrate prey items whereas the diet of the pelagic morph was strictly zooplankton. This indicates a more generalist feeding strategy in those charr with benthic morphology and a more specialist feeding strategy amongst those charr with characteristically pelagic morphology. This is unexpected, as generally, limnetic morphs have been found to be more flexible in foraging behaviour, due to the unstable temporal availability of zooplankton (Skulason et al. 1996). Laboratory studies of three-spined sticklebacks have also shown that the limnetic morphs are capable of feeding on both benthic invertebrates and zooplankton prey and generally have a more variable diet than benthic morphs (Day et al. 1994). The results shown here may however reflect seasonal changes in dietary composition as the charr from Coniston Water were caught in June, a time where there will be a relative abundance of zooplankton. It would therefore be surprising if generalistic feeders such as Arctic charr did not take advantage of an abundance of a prey item. The diet of Arctic charr is known to vary temporally in many populations and the diet of sympatric populations is known to overlap in some lakes (Svennig et al. 2007). A longer-term dietary study would be needed in order to determine seasonal variation in the diet of charr in Coniston Water. Benthic morphs may also be encouraged to feed on pelagic prey during the summer months by a reduction in the availability of benthic habitat. For example, increased productivity and stratification of the water column during the summer months can cause a reduction in oxygen concentrations in deep benthic waters. If this was to occur, in theory, Arctic charr may avoid foraging in deep waters (Mills et al. 1990, Jones et al. 2008). The oxygen concentrations in the profundal zone of Coniston Water have not been assessed but the lake is experiencing problems with mild eutrophication so it is possible that oxygen concentration may decline in the summer as has been observed in Windermere (Winfield 1992).

A longer-term assessment of diet may have been determined from carbon and nitrogen stable isotope signatures of white muscle tissue that would provide information over approximately six months. This, however, was not possible as muscle sample were not available and instead stable isotope analysis was carried out on scales. Since scales are grown throughout the individual's lifetime their carbon and nitrogen isotopic values provide an assessment of assimilated diet since first feeding. The results presented here show that variation in isotopic values of individuals within or between sub groups is limited. These results suggest little dietary segregation between the two morphs in Coniston, which is surprising given the degree of morphological segregation, although the dietary analysis does suggest the presence of a generalist benthic morph and a specialist zooplanktivorous morph. However, because the analyses were carried out on whole scales, they integrate across the fish's life time, while the dietary differences of trophic morphs are likely to be more clearly expressed across the outer portion of the scale, reflecting the time since developmental divergence.

As previously described, phenotypic variation in Arctic charr systems is either the result of phenotypic plasticity, where environmental factors influence juvenile behaviour and thus induce differences in morphology, or of heritable genetic differences, or of a combination of the two. Evidence for both sources of phenotypic variation within Arctic charr has been determined in laboratory breeding studies (Klemetsen et al. 2002, Adams and Huntingford 2004). Arctic charr are generally known to be generalist foragers with the capability to specialise where prey and habitat variability allow (Amundsen 1995). In the case of Coniston Water this divergence has taken place and two morphotypes occur in sympatry. Although their morphology is clearly linked to foraging in divergent habitats, the examination of diet suggests that neither morph utilises these habitats exclusively. This would suggest that the trophic polymorphisms observed are not exclusively caused by phenotypic plasticity within an individual's generation, as strong dietary segregation would be required to underpin the morphological divergence. It is beyond the scope of this study to provide conclusive evidence of heritable genetic basis of phenotype

although an assessment of the genetic divergence of morphotypes (Chapter Four) may give stronger support for this conclusion.

The different patterns of variation observed within these lakes in such close proximity to one another is not surprising as the degree of phenotypic segregation of Arctic charr within lakes is known to vary greatly in magnitude (Gislason et al. 1999, Jonsson and Jonsson 2001). The factors determining patterns of sympatric variation among lakes are in much debate although a link to niche availability and therefore lake morphometry has been presumed (Sandlund et al. 1992, Riget et al. 2000). Despite Windermere having a larger surface area both lakes have similar habitats available for habitation by Arctic charr, although Windermere now supports a much more complex fish community than Coniston Water, largely due to the introduction of species such as roach. Roach and other zooplanktivorous species cohabiting with Arctic charr in Windermere may have a competitive impact reducing the availability of the limnetic niche. The absence of competitors in Coniston Water, together with the longer-term stability of the fish community in this lake, could have allowed the charr to inhabit multiple niches resulting in the polymorphism observed.

Chapter 4 : Genetic differentiation among sympatric populations of Arctic charr in Windermere and Coniston Water

4.1. Introduction

4.1.1. Relationship between phenotypic variability and genetic differentiation

4.1.1.1. Evidence of genetic differentiation of sympatric morphs

Despite the extensive morphological variation between sympatric populations of Arctic charr records, genetic studies using markers such as allozymes and mitochondrial DNA (mtDNA) have shown only limited genetic differentiation between morphotypes within lakes (Kornfield et al. 1981, Danzmann et al. 1991, Hartley et al. 1992, Volpe and Ferguson 1996, Hendry and Kinnison 2001). Danzmann et al. (1991) used 46 restriction enzymes to analyse restriction fragment length poymorphisms in mtDNA of individuals from the four sympatric morphs of Arctic charr found in Lake Thingvallavatn, Iceland. Mitochondrial DNA has a higher mutation rate and smaller effective size than single copy nuclear genes so the probability of detecting differences is increased. Despite this, the study detected no significant genetic differentiation between morphs. Results from molecular genetic studies of other fish species e.g. the neotropical guppy, *Poecilia reticulata* (Reznick et al. 1997) also found little genetic divergence at mtDNA markers between ecologically and morphologically differentiated populations in sympatry, leading to the conclusion that such populations are not reproductively isolated (Orr and Smith 1998).

Relatively recent genetic techniques, however, use high-resolution molecular markers such as microsatellite loci, to detect small degrees of genetic differentiation between evolutionary young populations. Examples of genetic differentiation between landlocked populations of Arctic charr in Maine (Bernatchez et al. 2002) and between anadromous populations of Arctic charr in Canada (Bernatchez et al. 1998) have been shown using microsatellite loci. Significant genetic differentiation has also been found between sympatric morphs of Arctic charr within lakes. Gislason et al. (1999) studied variation at five microsatellite loci between sympatric morphs within five Icelandic lakes and found a range of different levels of

reproductive isolation from complete isolation between piscivorous and bethivorous morphs in Lake Galtaból and non-significant differentiation between limnetic and benthic morphs in Stóra Vioarvatn.

4.1.1.2. Origin of phenotypic and genotypic diversity

There are two possible scenarios for the origin of sympatric morphs within freshwater systems, the colonisation of one ancestral morph and the subsequent divergence of multiple morphs in sympatry or a double colonisation of morphs that diverged in allopatry. Empirical evidence shows a sympatric origin for divergence of some freshwater fish where morphs of the same species have repeatedly evolved locally (Taylor and Bentzen 1993), whereas an allopatric origin for currently coexisting morphs has been suggested for others, for example, lake whitefish (Bernatchez and Dodson 1990). In this study, Bernatchez and Dodson (1990) used thirteen restriction enzymes to analyse restriction fragment length polymorphisms (RFLP) in mtDNA of 156 individuals representing 13 populations throughout Eastern Canada and North Maine where morphs of lake whitefish occur in both sympatry and allopatry. The two morphs were from two separate lineages with differing distributions, only occurring together in certain lakes as a result of secondary contact during the last glaciation events. Studies have attempted to determine the likelihood of each scenario in Arctic charr using methods of both mtDNA sequence analysis and microsatellite analysis (e.g. Wilson et al. 1996, Gislason et al. 1999, Brunner et al. 2001).

4.1.2. Sympatric processes of genetic divergence

Genetic divergence between populations will occur when reproductive isolating mechanisms preventing gene flow between them are in place (see Chapter One for more detail). In the process of ecological speciation in sympatry these are usually based on individual behaviour. If different habitats or niches have sufficiently different selection regimes, local adaptation can develop despite substantial gene flow. Reproductive isolation then develops gradually as a by-product of habitat use (*divergence-with-gene-flow model*, reviewed in Rice and Hostert 1993). Links between ecological divergence and reproductive isolation have been demonstrated

experimentally in some species. For example, in mate choice experiments, limnetic and benthic morphs of three-spine sticklebacks will choose mates with similar body morphology to their own (Schluter 1995, 1996). In this case, positive assortative mating based on feeding morphology, results in the reproductive isolation of morphs. Other factors that may result in reproductive isolation include differences in spawning habitat between morphs, for example because of strong philopatry, different spawning seasons (allochrony) (Skulason 1992, Jonsson 1996) and environmental selection against hybrids, known as reinforcement (Wood and Foote 1996).

The role of natural selection in the generation of reproductive isolation is highly controversial, although in theory it is possible that selection will increase sexual isolation between two sympatric populations by selecting against maladaptive hybrids (Ďobzhansky 1937). Evidence of this reinforcement comes from observations of sympatric populations that have shown greater sexual isolation than allopatric populations of the same species (Coyne and Orr 1999, Noor 1999). Individuals that preferentially mate with conspecifics produce fitter offspring than those that mate at random, thus selection for positive assortative mating will occur (*reproductive character displacement*, Howard 1993). Empirical evidence for reinforcement has been documented for sympatric three-spine sticklebacks species pairs that will hybridise and produce fertile offspring in the laboratory and some hybrids have been observed in the wild. In this case F_1 hybrids suffer a foraging disadvantage relative to their parents caused by their intermediate morphology (Rundle and Schluter 1998). Reinforcement can also act without postzygotic isolation when hybrids have difficulty securing mates (Coyne and Orr 1999). Despite observational evidence, it is also very difficult to distinguish reinforcement from other possible ecological causes of character displacement (Howard 1993), and ecological field studies are lacking (Noor 1999).

Spawning behaviour and mate selection is highly sophisticated in many freshwater fish species, involving colour patterns, olfactory and visual cues. Assortative mating in many species of freshwater fish, for example three-spine sticklebacks (Gudbjorg et

al. 2006, Vines and Schluter 2006) and African cichlids (Gavrilets et al. 2007) has been confirmed by female mate choice experiments in the laboratory, and often leads to reproductive isolation (Skulason et al. 1999). In salmonids, mate selection is complex and often related to body size and spawning colours (Fleming and Reynolds 2003). Some examples of sympatric morphs of Arctic charr differ in size at sexual maturity and could therefore theoretically lead to size assortative mating, which has been observed for some salmonid species, including Japanese charr (*Salvelinus leucomaenis*) and Atlantic salmon (*Salmo salar*) (Maekawa et al. 1994, Taggart et al. 2001) but this has not been tested in Arctic charr.

Arctic charr are localised benthic spawners and sexually mature fish are known to have a very strong homing instinct (Behnke 1972). Much literature is available on the subject of natal homing (philopatry) in salmonids, but is largely confined to that of anadromous populations, as the two traits are often closely associated, and studies specific to Arctic charr are limited. Anadromous individuals, migrating to the sea in order to feed over the summer months will then return to their natal river to spawn. This behaviour has clear selective advantages as fish are returning to spawning areas of known reproductive success (McDowall 2001). On the other hand it is also evident that many individuals 'stray' and fail to return to their natal spawning grounds (Quinn and Dittman 1990). This also has advantages in allowing the colonisation of new favourable habitats and allows the spread of advantageous genotypes (McDowall 2001). Although it is understood that many individuals are capable of returning to their natal river for spawning, homing patterns at smaller spatial scales, for example to specific spawning sites, within populations are less well known (Neville et al. 2006). Mark-recapture and tagging studies have indicated strong fine-scale natal homing in anadromous sockeye salmon (*Oncorhynchus nerka*) within river basins (Stewart et al. 2004).

Homing patterns in relict lake-resident non-anadromous salmonids are also poorly understood but it is hypothesised that some individuals will retain the instinct to return to natal spawning grounds despite remaining locally for the whole of the life cycle. Therefore, where morphological divergence is associated with strong

philopatry, distinct spawning grounds can develop between morphs, a process that could genetically isolate populations in sympatry (Adams et al. 2006). This is especially likely if available spawning habitats are discrete. Rates of gene flow as low as one migrant per generation are thought to be enough to prevent a loss of genetic diversity through drift whilst allowing the population to respond to local selection pressures (Mills and Allendorf 1996). Strong spawning site fidelity would therefore reduce gene flow enough to allow populations to diverge through local adaptation or by drift.

Most often, amongst populations, Arctic charr spawn in the autumn in order to coincide with availability of resources for the first feeding of juveniles in the following spring. Sympatric morphs of Arctic charr have been known to diverge, spawning later or earlier in the year (Westgaard et al. 2004). The mechanisms involved in the evolution of temporal spawning variation, allochrony, are unclear but several mechanisms have been suggested. Seasonal changes in the availability of prey specific to each morph may alter spawning times by affecting annual patterns of gonad production to tie in with food availability (Skulason et al. 1989). For example, benthic food types may be available throughout most of the year whereas in temperate climates zooplankton abundance will peak during spring. Other theories suggest that breeding time is genetically controlled and its divergence in sympatric populations evolved via pleiotropy, for example if phenotype and breeding time are controlled by the same gene, or by genetic hitchhiking with selected traits (Rice and Hostert 1993). Disruptive selection acting directly on variation in breeding time can also cause divergence in breeding season. In this case positive assortative mating will occur when early breeders mate with early breeders and late breeders mate with late breeders. In anadromous salmonids variation in maturation and migration rates may lead to divergence between those individuals that mature earlier in the season and therefore return to spawn first and those that mature later and therefore return to spawn later in the year (Hendry 2001).

Frost (1965) described 13 probable spawning sites for Arctic charr in Windermere. These included five autumn and two spring sites in the north basin and four autumn

and two spring sites in the south basin (see Chapter Three Figure 3.1), although it is unclear as to whether these are all utilised by Arctic charr today. There are also sites that are utilised both in the autumn and spring including Rough Holme in the north basin (J. Fletcher pers. comm.) and Rawlinson's Nab in the south basin (Partington and Mills 1988). Kipling and Le Cren (1984) found that of 2066 spawning fish tagged on four spawning sites, 578 (28 %) were recaptured in subsequent years. Of these fish, 99 % were captured at the same spawning site on which they were originally tagged, and some individuals were caught in three consecutive years on the same spawning ground. Although it is not certain that individuals are returning to their natal site to spawn, or that they do not use multiple spawning sites, this evidence suggests this is highly likely and such strong site fidelity should result in genetic sub-structuring of populations based on spawning ground.

Previous attempts to assess genetic differentiation between the Windermere spawning groups have concentrated on isozyme electrophoretic techniques (Child 1984, Partington and Mills 1988). Partington and Mills (1988) assessed the differentiation between spawning populations in both basins of Windermere, UK, by comparing allele frequencies for serum esterase and for skeletal muscle malate dehydrogenase. Their results showed no significant differences in allele frequencies between the autumn- and spring-spawning populations in either the north or the south basin but this type of genetic analysis has very low resolution and these loci may have been affected by selection. They did however find a significant difference in the frequencies of alleles at both loci between basins for the autumn-spawning fish but not between the basins for the spring-spawning fish, concluding that genetic divergence had occurred between basins in the autumn-spawning population only and there was no divergence based on spawning season.

4.1.3. Chapter Aims

The genetic differentiation of sympatric populations in both Windermere and Coniston Water was assessed using microsatellite markers to test the following hypotheses.

1. Population sub-structuring in Windermere is based on spawning behaviour.

2. Gene flow between the north and south basins of Windermere is restricted. Therefore the autumn and spring spawning populations in both basins represent four distinct sub-populations.
3. In Coniston Water, genetic variation is correlated with the observed phenotypic variation

The results are interpreted in the context of the underlying evolutionary mechanisms in order to provide insight into the reproductive isolation and genetic divergence of sympatric populations of Arctic charr.

4.2. Methods

4.2.1. Microsatellite analyses

Microsatellites, repeating units of DNA of one to six base pairs in length, show high levels of polymorphism, and high heterozygosity. They are therefore a powerful molecular marker providing useful information in studies that focus on DNA variation for population structure (Schlotterer 1998). They are neutral markers, and although they sometimes occur linked with selected loci, they are for the most part unaffected by natural selection, and thus provide information on genetic variation caused by alternative evolutionary processes such as gene flow and genetic drift.

4.2.1.1. Samples obtained

Samples for genetic analysis were obtained for all fish from Windermere and Coniston Water for which morphological assessment was made in Chapter Three. Information on the sampling technique is therefore provided in Chapter Three. The locations and depths of spawning grounds are shown in Figure 4.1. Fin clips were taken from a further 36 fish from the north Thompson Holme Autumn spawning site in the North basin, which were then released back into the lake in accordance with Environment Agency licensing restrictions at this site. Total sample sizes for each site are given in Table 4.1. It was necessary to pool data from multiple sites so as to attain workable sample sizes for statistical analysis in all but the spring south sub-group, where only one site was sampled.

In each case the adipose fin was removed from the fish using scissors and placed in labelled tubes of 20 % salt saturated DMSO solution. The scissors were cleaned

using ethanol after each sample to avoid contamination. The samples were then stored at –20 °C to minimise degradation of the DNA.

Table 4.1. Total sample sizes of fish used in the genetic analysis of Windermere populations

Site Name	Depth (m)	Basin	Spawning period	Number of fish	Totals	
South Rawlinsons Nab	20	South	Spring	28	28	71
Holbeck	10	North	Spring	30	43	
Rough Holme	3	North	Spring	13		
Grass Holme	4	South	Autumn	18	31	92
Sewage works	6	South	Autumn	13		
High Wray Bay	4	North	Autumn	8	61	
North Thompson Holme	3	North	Autumn	47		
Rough Holme	3	North	Autumn	6		

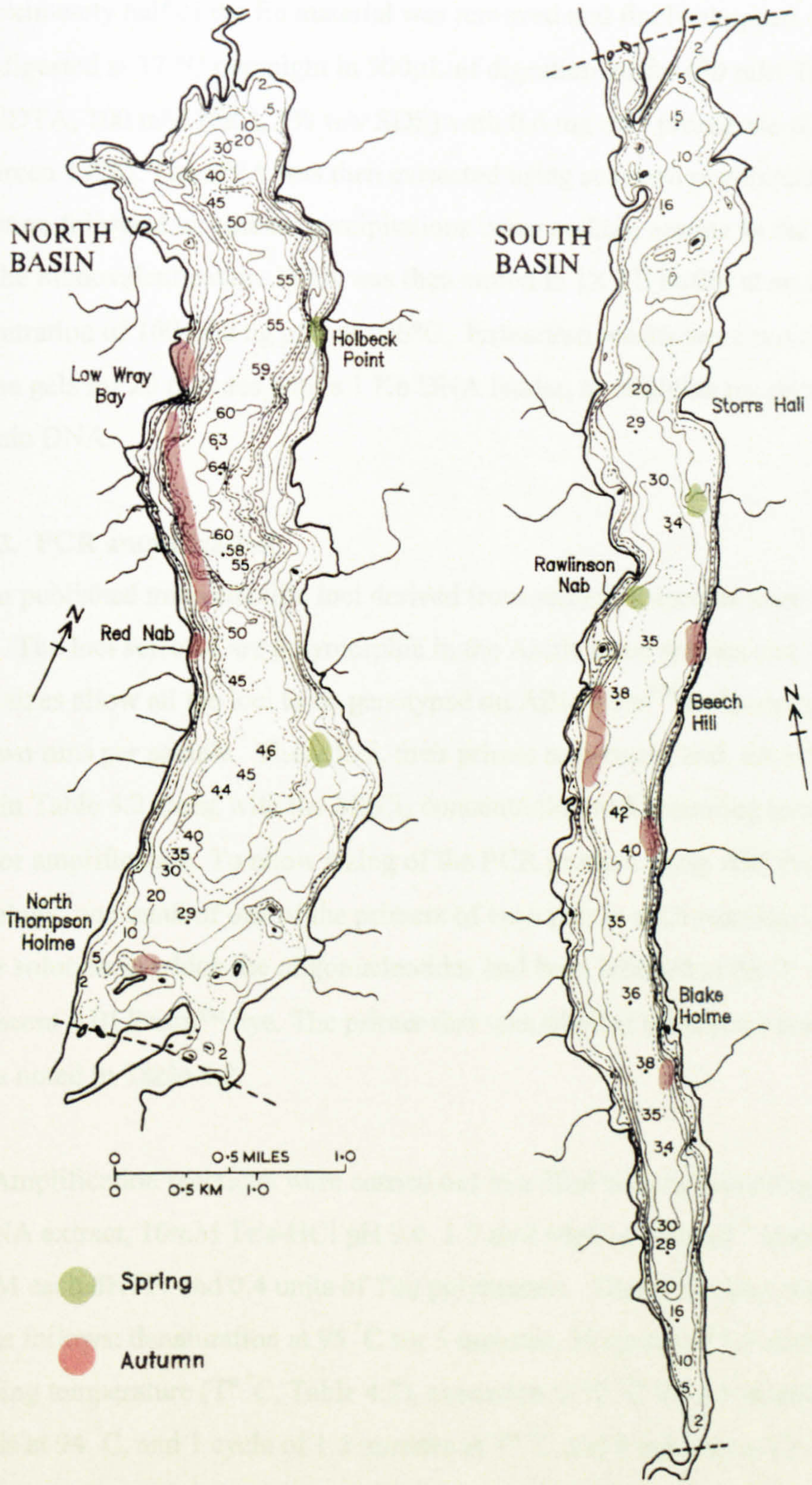


Figure 4.1. Location and depth of spawning grounds in Windermere, adapted from Frost (1965) and Ramsbottom (1976).

4.2.1.2. DNA extraction from tissue samples

Approximately half of the fin material was removed and finely chopped. Samples were digested at 37 °C overnight in 500µL of digestion buffer (50 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl, 1% w/v SDS) with 0.6 mg ml^{-1} proteinase K (Hoelzel and Green 1998). The DNA was then extracted using standard phenol/chloroform extraction followed by ethanol precipitations using sodium acetate as the source of Na^+, the monovalent cation. DNA was then stored in 1X TE buffer at an approximate concentration of 100-200 ng μL^{-1} at –20°C. Extraction results were run on 1.2 % agarose gels for 20 minutes with a 1 Kb DNA ladder, to establish the presence of genomic DNA.

4.2.1.3. PCR amplification

Eleven published microsatellite loci derived from salmonid species were used in this study. The loci selected are polymorphic in the Arctic charr species and were chosen as the sizes allow all the loci to be genotyped on ABI Prism™ technology gels in only two runs per sample. These loci, their primer sequences, and, the references are listed in Table 4.2 along with the $MgCl_2$ concentration and annealing temperatures used for amplification. To allow sizing of the PCR product using ABI Prism™ technology, one tenth of one of the primers of each pair in each reaction was from a primer solution in which the oligonucleotides had been labelled at the 5' end with a fluorescent ABI Prism™ dye. The primer that was labelled in each set and the dye used is noted in Table 4.2.

PCR Amplification reactions were carried out in a 20µl volume containing 0.5 µl of the DNA extract, 10mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 10ng μl^{-1} labelled primer, 0.2 mM each dNTP, and 0.4 units of *Taq* polymerase. Thermocycling conditions were as follows: denaturation at 95 °C for 5 minutes, 35 cycles of 1.5 minutes at annealing temperature (T^a °C, Table 4.2), extension at 72 °C for 1.5 minutes, and 45 seconds at 94 °C, and 1 cycle of 1.5 minutes at T^a °C and 8 minutes at 72 °C (extension). Exceptions to these conditions were used for loci Smm10, Smm24, SalD23 and SalD30. Cycle conditions for Smm10 and Smm24 were: denaturation at 92 °C for 2 minutes, 30 cycles of denaturation at 92 °C for 15 seconds, 15 seconds at

T^a °C and extension at 72 °C for 30 seconds, followed by a final extension at 72 °C for 10 minutes. Cycle conditions used for SalD23 and SalD30 were: denaturation at 94 °C for 2 minutes, 30 cycles of denaturation at 94°C for 30 seconds, 30 seconds at T^a °C and extension at 72 °C for 45 seconds, followed by a final extension at 72 °C for 10 minutes.

Table 4.2. Microsatellite loci, their primer sequences and annealing temperatures (T^a °C). In each case the forward primer was fluorescently labelled with the dye indicated.

Locus	Primer Sequence (5'–3')	Reference	T^a (°C)	Allele Size (bp)	Repeat Motif	Dye
Sfo334 lav	GGA TTA ACA GAA GGT TAC TG	Perry et al (2005)	52	216-296	$(TG)_{58}G_4(TG)_{27}(CGTG)_8$	FAM
	CTT CGT ATT CTT CAT TGT GC					
Smm 17	AAG GAT GGT GAG GAC AAT ACA	Crane et al (2004)	58	107-131	$(CA)_{29}$	FAM
	ACC TTG AGA AAT CTA TAT GTG GTC TA					
Sal O23	TCT GAA TGC AGC CCC ACA G	McGowan et al (2004)	60	210	$(TG)_{60}$	HEX
	TTC AAG CCA AAG GAC ACA TGG					
Ssa 85	AGG TGG GTC CTC CAA GCT AC	O'Reilly et al (1996)	60	161-211	$(GT)_{14}$	NED
	ACC CGC TCC TCA CTT AAT C					
Smm 10	AAA ATG TCT CCC CTC CCT CTC	Crane et al (2004)	55	150-170	$(TCCA)_{16}$	FAM
	TCC CTA ACA TAA CAA GTT TTC ATC CT					
Sal J 81	CAG CAT AAT CAC TCC CGC	McGowan et al (2004)	52	125-149	$(GT)_{33}$	HEX
	GAA AGC TAC CTT GCG TGC					
Sal D 30	TTT GTT GTT ATG ACT CTG CG	McGowan et al (2004)	55	158-178	$(AC)_{52}$	FAM
	CAA GCA GAA TCG TTT GGT C					
Omm1302	AGC CAG CCA ATT AAT ACC CTG	Palti et al (2002)	58	184-212	CATC/CT	HEX
	TTC TGT GTG GCC TAA ACC TT					
Omm1377	TGT GTC TCT CAA TGC GAC CTG	Rodiguez et al (2003)	58	258-268	(CA)* imperfect repeat	FAM
	GGC AAA ACT CCA CGG ACT GTA					
Mst 85	GGA AGG AAG GGA GAA AGG T	Presa and Guoymard (1996)	52	202-238	$(CT)_{22}$	NED
	GGA AAA TCA ATA CTA ACA A					

4.2.1.4. Interpretation of results

PCR products were run on 2 % agarose gels for 20 minutes with a 100bp DNA ladder to establish the presence and size of the DNA. DBS Genomics (Durham University) ran them on a 377 ABI polyacrylamide slab gel automated sequencer. As mentioned in the earlier section on PCR, each product had been labelled by the use of a fluorescent labelled primer, allowing the product to be detected by the sequencer. ABI Prism™ fluorescent labels of FAM, HEX, and NED were used. The PCR products were then added in specific amounts (0.2 μL for FAM dyed PCR products, 0.3 μL for the HEX dyed products and 0.4 μL for the NED dyed products) to a 1.625 μL mixture of ABI loading buffer containing ROX labelled DNA size ladder to allow the sizing of the PCR products. Sets of loci were assembled taking care not to overlap allele sizes on the same given dye before being run together on the 377 ABI sequencer. Visualization of PCR product sizes to a resolution of 1 bp was possible on a chromatogram produced by analysis of the output of the automated sequencer using ABI Genescan™ and Genotyper™ software.

Microsatellite alleles were considered reliable and used in the analysis if the peaks met certain criteria. First, the highest amplitude peak, used as the allele size, was only considered valid if it had an amplitude higher than 50 on the chromatogram. Most alleles were well above this amplitude, but any peaks below 100 were duplicated before use in the analysis. Second, alleles deemed reliable had to show the expected signature structure. Each locus showed a pattern in the shape and prominence of the stutter peaks associated with an allele, and any peaks not showing this pattern were considered to be background 'noise' in the chromatogram or unspecific amplification.

4.2.2. Statistical analyses

4.2.2.1. Microsatellite loci analysis

During the process of identification and amplification using primers and polymerisation chain reaction errors can occur. Alleles may fail to amplify i.e. null alleles (Wandeler et al. 2003) or large alleles may not amplify as efficiently as smaller alleles, known as large allele dropout. Slight changes to allele sizes during

amplification, known as stuttering, can lead to scoring errors. Before proceeding with analysis, these errors were investigated using the software Micro-checker (Van Oosterhut et al. 2004). Micro-checker calculates the probabilities for the observed number of homozygotes at each locus to see if there is homozygote excess, which could be evidence of null alleles. Stuttering is suggested when there is a deficiency of heterozygotes with alleles differing in size by one nucleotide repeat, and large allele dropout is indicated by a relative excess of large homozygotes.

Polymorphism was estimated as the number of alleles per locus, number of private alleles per putative population, allelic richness, and observed and expected heterozygosity. Deviation from Hardy-Weinburg equilibrium was tested using a method analogous to Fisher's exact test using a modified version of the Markov-chain method (Guo and Thompson 1992), implemented in ARLEQUIN 2.000 (Schneider et al. 2000). Allelic richness for each locus and each population was calculated using the program FSTAT 2.9.3 (Goudet 2001) and differences among populations was tested using a Kruskal-Wallis test.

Tests for linkage disequilibrium were carried out for each pair of loci using GENEPOP 3.4 (Raymond and Rousset 2001). This determines whether associations exist between pairs of loci by using Fisher's exact probability test (Guo and Thompson 1992).

An assessment of the degree of random mating was made using Wright's F_{IS} (Wright 1965). Non-random mating is a cause of reduced heterozygosity at any given loci therefore the degree of non-random mating within a population can be assessed by comparing the observed and expected heterozygosity values. F_{IS} was calculated using the program FSTAT 2.9.3 (Goudet 2001).

The levels of differentiation between pairs of populations within Windermere were first quantified by estimates of pairwise fixation indices based on the infinite allele model, F_{ST} (Weir and Cockerham 1984), in the program ARLEQUIN 2.000 (Schneider et al. 2000). Pairwise differentiation was also estimated using mutational differences

among alleles, assuming the stepwise mutation model by computing pairwise standardised Rho_{ST} estimates, using the program RSTCALC 2.2 (Goodman 1997). Statistical significance was calculated by permutation tests with bootstrapping to provide 95 % confidence levels with 1,000 iterations.

The extent of population differentiation was further assessed by performing a factorial correspondence analysis (FCA) in the program Genetix 4.0 (Belkhir 1999). The use of FCA to analyse genetic data has been described by (She et al. 1987) and is used in order to give a visual representation of individuals clustering on the basis of their relative allele frequencies. Genetic data are transformed into a contingency table where each sample is described by the allelic frequencies at each locus. The χ^2 distance centred on the marginal distribution of the contingency table is used to measure the relatedness between any two samples in the k-dimensional space, where k is the number of alleles.

Gene flow between spawning populations within each basin and between basins was estimated using the coalescent-based Monte Carlo Markov chain (MCMC) method implemented in MIGRATE (Beerli and Felsenstein 1999). For each pair of populations, geneflow was estimated as the effective number of migrants per generation from population y to population x, $N_{ex}m_{yx}$, where N_e is effective population size and m is migration rate. Data were analysed under an assumed Infinite Allele model of microsatellite mutation. The MCMC parameters used were as follows: 20 short chains with 500 geneologies followed by five long chains with 50,000 geneologies and a burn-in of 10,000. Multiple runs were performed to test the convergence of parameter estimates obtained for each population.

4.2.2.2. Relationship between morphological variables and genetic variation

In order to examine whether environment- or morphology-related variables explained any of the observed genetic variation among populations of Arctic charr, partitioning of a genetic distance matrix was performed using distance based redundancy analysis (dbRDA, McArdle and Anderson 2001). This is a form of multivariate multiple regression which can be performed directly on a distance matrix. The matrix of

individual pairwise Chord distances, (D_{CE}; Cavali-Sforza and Edwards 1967), was produced in the program Populations (Langella 2001) using microsatellite DNA allele frequencies. The categories, spawning season and the depth of where the fish were caught on the spawning grounds were included as predictors of spawning behaviour of individuals. Then ten standardised morphological measurements (details of which are found in Chapter Three) were considered separately and in sets on the basis of head morphology, body morphology and fin lengths (Table 4.3). Body weight was included separately. The relationship between the distance matrix and each of the predictor variables (Table 4.3) was analysed for individuals grouped by basin, using dbRDA in the program DISTLM*forward* (Anderson 2003) For these analyses *P*-values were obtained using 10,000 unrestricted but simultaneous permutations of the rows and columns of the distance matrix. This examined the extent to which any of the predictor variables explains significant genetic variability between individuals over and above that explained by spawning season classification alone.

Table 4.3. Sets of predictor variables used in the distance based redundancy analysis

Set	Predictor variable
Spawning Season	Spring (S) or Autumn (A)
Spawning Depth	Depth samples caught in metres
Head Morphology	Head length
	Head depth at operculum
	Head depth at eye
	Lower jaw length
	Eye diameter
Body Morphology	Total Length
	Body width
	Caudle Peduncle width
Fin lengths	Pectoral fin length
	Pelvic fin length
Weight	Total body weight

4.3 Results

4.3.1. Genetic differentiation between spawning populations in Windermere

4.3.1.1. Genotyping errors, test for Hardy Weinburg equilibrium and genetic diversity

Homozygote excess was found for the locus Sfo334, indicating null alleles or large allele dropout. This locus was therefore removed from further analysis. No evidence of genotyping errors was found for any further loci; however, heterozygote deficiency compared against the Hardy-Weinburg equilibrium expectations was found for the locus Mst85. This only occurred in one sub-population and its omission did not significantly change the pattern of differentiation of the population so it was retained for the analysis. No significant heterozygote excess was found for any locus. Each pair of loci was tested for linkage disequilibrium and genotypic independence was confirmed. Mean allelic richness was not significantly different among populations ($\chi^2 = 0.702$; df = 3). The degree of random mating (F_{IS}) was based on observed and expected heterozygosity values for each locus. Large values of F_{IS} for a particular locus can suggest homozygous excess at that locus and is evidence of non-random mating. The spring north sub group showed the highest mean value of F_{IS} over all loci (0.03). A summary of the statistics computed on the microsatellite genotype data is presented in Table 4.4.

Table 4.4. Number of alleles (A), private alleles, allele size range, expected (H_e) and observed (H_o) heterozygosity for each sub-group (autumn south, AS; autumn north, AN; spring south, SS; spring north, SN) at each microsatellite locus. Asterisks indicate those loci with a p-value <0.001 (Bonferroni correction applied) when tested for heterozygote deficiency.

		AS	AN	SS	SN
Smm17	N	24	61	28	43
	A	7	7	5	7
	Private A	-	1	-	-
	Allelic richness	6.92	5.88	4.82	5.85
	Size range	111-125	109-125	111-125	111-125
	H_o	0.750	0.845	0.857	0.766
	H_e	0.788	0.767	0.691	0.752
	F_{IS}	0.035	-0.103	-0.246	-0.018
Smm10	N	24	61	28	43
	A	5	5	5	6
	Private A	-	-	-	1
	Allelic richness	4.96	4.02	4.77	5.01
	Size range	147-159	147-163	147-163	143-163
	H_o	0.833	0.704	0.654	0.702
	H_e	0.676	0.645	0.661	0.689
	F_{IS}	-0.238	-0.093	-0.018	-0.02
Ssa85	N	24	61	28	43
	A	12	19	17	16
	Private A	-	1	2	-
	Allelic richness	11.91	14.07	15.89	13.17
	Size range	159-199	159-205	159-205	159-205
	H_o	0.833	0.843	0.821	0.702
	H_e	0.856	0.890	0.940	0.836
	F_{IS}	0.026	0.053	0.122	0.160
Omm1302	N	24	61	28	43
	A	6	7	9	7
	Private A	-	-	3	1
	Allelic richness	5.96	5.75	8.73	6.10
	Size range	183-207	187-207	187-211	183-207
	H_o	0.667	0.732	0.923	0.915
	H_e	0.706	0.724	0.834	0.764
	F_{IS}	0.029	-0.012	-0.124	-0.201
SalJ81	N	24	61	28	43
	A	5	6	4	7
	Private A	-	-	-	1
	Allelic richness	4.96	5.08	3.82	5.84
	Size range	135-145	135-145	135-141	135-145
	H_o	0.917	0.732	0.643	0.745
	H_e	0.696	0.720	0.649	0.734
	F_{IS}	-0.326	-0.018	0.009	-0.014

Table 4.4. Continued

Omm1377	N	24	61	28	43
	A	5	8	5	7
	Private A	-	2	-	1
	Allelic richness	5.00	6.01	4.97	6.17
	Size range	255-265	251-267	255-265	251-265
	H_o	0.833	0.823	0.714	0.766
	H_e	0.753	0.752	0.744	0.771
	F_{IS}	-0.011	-0.110	0.037	-0.003
Mst85	N	24	61	28	43
	A	12	12	10	18
	Private A	1	2	-	2
	Allelic richness	14.71	11.39	9.57	13.70
	Size range	202-262	202-254	202-242	202-254
	H_o	0.833	0.662	0.607	0.681*
	H_e	0.826	0.756	0.812	0.799
	F_{IS}	-0.030	0.117	0.245	0.146
SalO23	N	24	61	28	43
	A	16	24	15	26
	Private A	-	2	1	3
	Allelic richness	18.00	16.89	14.31	19.08
	Size range	209-265	207-265	207-257	207-263
	H_o	0.913	0.930	0.857	0.830
	H_e	0.936	0.927	0.906	0.946
	F_{IS}	0.025	-0.004	0.048	0.116
SalD30	N	24	61	28	43
	A	11	17	14	16
	Private A	-	2	1	3
	Allelic richness	12.87	14.46	13.57	12.77
	Size range	166-190	166-200	170-202	158-200
	H_o	0.875	0.929	1.00	0.851
	H_e	0.917	0.923	0.959	0.899
	F_{IS}	0.043	-0.006	-0.081	0.054
Total	**Mean A**	**8.78**	**11.67**	**9.33**	**12.22**
	Mean A. Rich ± SD	**9.48±4.97**	**9.28±4.90**	**8.94±4.68**	**9.74±5.03**
	Mean H_o ± SD	**0.83±0.08**	**0.80±0.10**	**0.79±0.14**	**0.78±0.08**
	Mean H_e ± SD	**0.79±0.10**	**0.79±0.10**	**0.80±0.12**	**0.80±0.08**
	Mean F_{IS}	**-0.051**	**-0.016**	**0.004**	**0.030**

4.3.1.2. Population differentiation and gene flow

Patterns of genetic differentiation among pairwise populations using F_{ST} and Rho_{ST} values are displayed in Table 4.5. The F_{ST} values were low but significant between all spawning populations in Windermere whereas the Rho_{ST} values were low but significant in comparisons between all populations except for between autumn and spring populations in the north basin. The highest value occurred in the comparison between autumn south and spring south populations for both F_{ST} (0.0143) and RhoST (0.052). The comparisons between basins within the same spawning seasons gave similar values of both F_{ST} (0.0064 for autumn *cf.* 0.0068 for spring) and Rho_{ST} (0.021 for autumn *cf.* 0.020 for spring). The lowest values occurred in the comparisons between autumn and spring spawning populations in the north basin for both F_{ST} (0.005) and Rho_{ST} (-0.002).

Table 4.5. Pairwise F_{ST} (below diagonal) and Rho_{ST} (above diagonal) between spawning populations in Windermere, UK (autumn south, AS; autumn north, AN; spring south, SS; spring north, SN). Asterisks indicate significant values p<0.05, Bonferroni correction applied)

	AS	AN	SS	SN
AS		0.021*	0.052*	0.022*
AN	0.0064*		0.020*	-0.002
SS	0.0143*	0.0091*		0.020*
SN	0.0100*	0.0055*	0.0068*	

The factorial correspondence analysis (FCA) showed that despite some overlap, individuals clustered with others from the same spawning population on the basis of allelic frequency (Figure 4.2). The north basin populations of autumn and spring spawners showed a large degree of overlap, whereas the south basin populations of autumn- and spring-spawning fish showed the greatest degree of separation between populations. Both spawning populations are also separated to a lesser degree by

basin along FCA axes; spring spawning fish at FCA1 and autumn spawning fish at FCA2.

The pattern of genetic differentiation is confirmed by the pattern of gene flow estimates between the spawning populations (Table 4.6). The effective number of migrants was greatest between the autumn and spring spawning populations in the north basin, which coincides with the low values of F_{ST} and Rho_{ST}, although this movement was greater from the spring population to the autumn population. Directional gene flow from the north basin to the south basin can also be seen. In both spring and autumn spawning populations gene flow was greater from the north basin to the south basin than from the south basin to the north basin (SN→SS, 4.172 > SS→SN, 2.849 and AN→AS, 3.31 > AS→AN, 1.355). Gene flow between autumn and spring spawning populations in the south basin was low in both directions, corresponding to the higher values of F_{ST} and Rho_{ST}.

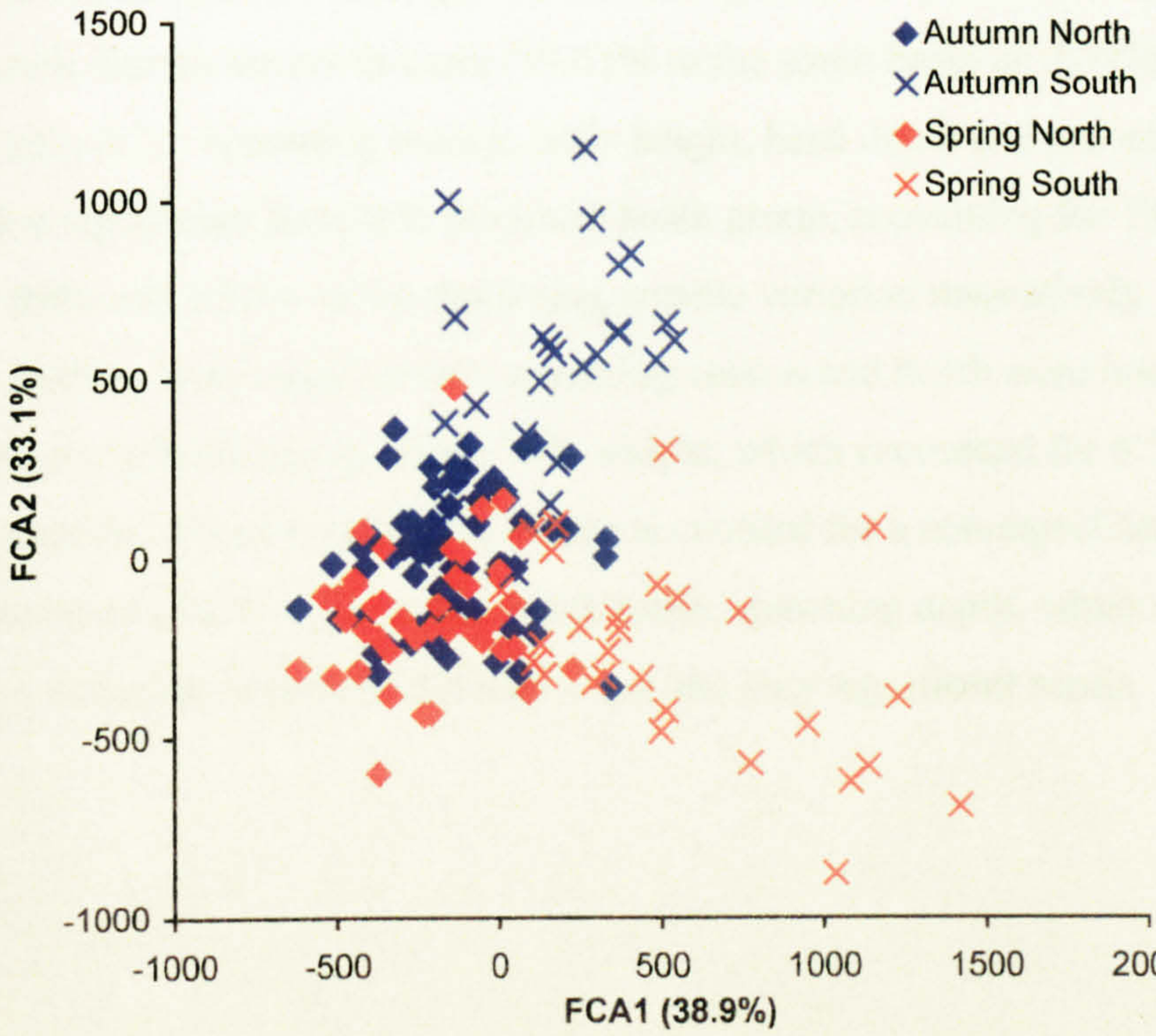


Figure 4.2. Genetic differentiation among Arctic charr spawning populations in Windermere, UK, based on factorial correspondence analysis (FCA) of alleleic frequency at 9 microsatellite loci. The amount of variation explained by each axis is given in parenthesis.

Table 4.6. Estimates of effective number of migrants $N_{ex}m_{yx}$ per generation between spawning populations in Windermere (autumn south, AS; autumn north, AN; spring south, SS; spring north, SN). 95 % confidence limits are given in parenthesis.

Receiving population (x)	Migrant source population (y)			
	AS	AN	SS	SN
AS		3.31 (2.86, 3.73)	2.494 (2.13, 2.72)	1.936 (1.43, 2.19)
AN	1.355 (1.09, 1.54)		1.347 (1.11, 1.60)	5.272 (4.65, 5.90)
SS	3.164 (2.78, 3.43)	4.021 (3.54, 4.35)		4.172 (3.64, 4.53)
SN	1.793 (1.50, 2.13)	2.367 (1.93, 2.63)	2.849 (2.40, 3.10)	

4.3.1.3. Relationship between genetic variation and morphology in Windermere

The individual predictor variable that had a significant relationship with genetic distances of Arctic charr in both the south and north basins was the depth at which an individual spawns, although this factor explained a lot more of the variation in the south than in the north basin (19.61% in the south basin *cf.* 2.91% in the north basin; Table 4.7). Spawning season, body height, head depth and pectoral fin length were also significant factors in the south basin group, accounting for 19.61%, 9.72%, 5.86% and 5.95% of the remaining genetic variation respectively. When predictor variables were tested in sets, spawning season and depth were both still significant in the south basin group, along with weight, which accounted for 8.53% of the variation. Head measurements also accounted for a non-significant proportion of the variation (Table 4.8). In the North basin, spawning depth, which explains 2.08% of the variation in genetic distances, was the only significant result.

Table 4.7. Results of sequential tests of each individual predictor variable using dbRDA multivariate F-statistic in Windermere Arctic charr from each basin.

South basin				North basin			
Set	F	*P*	% Variation	Set	F	*P*	% Variation
Spawning depth	**11.464**	**0.0001**	**19.61**	**Spawning depth**	**2.222**	**0.0007**	**2.91**
Spawning season	**11.463**	**0.0001**	**19.61**	PEC	1.329	0.126	1.74
Weight	1.844	0.197	3.78	HDO	1.210	0.225	1.57
PEC	2.659	0.083	5.36	Weight	1.159	0.267	1.51
PEL	**2.974**	**0.046**	**5.95**	PEL	1.071	0.378	1.39
BH	**5.060**	**0.001**	**9.72**	LJ	1.100	0.333	1.43
CP	1.805	0.206	3.7	BH	1.006	0.464	1.30
HL	1.657	0.238	3.41	CP	0.963	0.513	1.25
HDO	**2.928**	**0.037**	**5.86**	HL	0.948	0.532	1.23
ED	1.488	0.295	3.07	Spawning season	0.970	0.517	1.26
LJ	0.987	0.490	2.06	ED	0.848	0.672	1.10

Table 4.8. Results of sequential tests of predictor variables in sets using the dbRDA multivariate *F*-statistic in Windermere Arctic charr from each basin.

South basin				North basin			
Set	*F*	*P*	% Variation	Set	*F*	*P*	% Variation
Spawning depth	**11.464**	**0.0001**	**19.61**	**Spawning depth**	**1.591**	**0.036**	**2.08**
Head morphology	1.737	0.107	13.78	Head morphology	1.211	0.150	3.20
Weight	**2.936**	**0.040**	**8.53**	Weight	0.992	0.492	2.62
Spawning season	**4.890**	**0.012**	**6.47**	Spawning season	0.981	0.491	1.28
Fin lengths	1.923	0.209	4.86	Fin lengths	1.073	0.338	2.83
Body Morphology	1.478	0.335	3.64	Body Morphology	1.211	0.150	3.20

4.3.2. Genetic differentiation between morphotypes in Coniston Water

4.3.2.1. Genotyping errors, test for Hardy-Weinburg equilibrium and genetic diversity

Homozygote excess was found for the locus Smm10, indicating null alleles or large allele dropout. This locus was therefore removed from further analysis. No evidence of genotyping errors was found for any further loci. However, heterozygote deficiency compared against the Hardy-Weinburg equilibrium expectations was found for loci Smm 17 and SalO23. Further analysis was done both with these loci included and excluded. MST-85 was also excluded from the analysis due to problems with PCR amplification at this locus resulting in too few individuals genotyped. Each pair of loci was tested for linkage disequilibrium and genotypic independence was confirmed. Mean allelic richness was not significantly different among populations ($\chi^2 = 0.008$; df = 1). The pelagic sub group showed the highest mean value of F_{IS} over all loci (0.081). Table 4.9 summarises the results of the statistical analyses performed on the genotypic data for the Coniston Water morphotypes.

Table 4.9. Number of alleles (A), private alleles, allele size range, expected (He) and observed (Ho) heterozygosity for each sub-group at each microsatellite locus. Asterisks indicate those loci with a p-value <0.001 (Bonferroni-correction applied) when tested for heterozygote deficiency.

		Pelagic Morph	Benthic morph
Smm17	N	22	31
	A	5	6
	Private A	-	1
	Allelic richness	5.56	5.41
	Size range	109-117	109-129
	Ho	0.727	0.452*
	He	0.738	0.639
	F_{IS}	-0.015	0.258
Smm10	N	22	31
	A	4	5
	Private A	-	1
	Allelic richness	4.38	4.50
	Size range	151-163	151-163
	Ho	0.136*	0.323
	He	0.402	0.320
	F_{IS}	0.617	0.004
Ssa85	N	22	24
	A	10	9
	Private A	2	1
	Allelic richness	9.13	9.20
	Size range	159-199	
	Ho	0.909	0.958
	He	0.856	0.867
	F_{IS}	-0.052	-0.111
Omm1302	N	22	31
	A	6	6
	Private A	-	-
	Allelic richness	5.48	5.57
	Size range	191-211	191-211
	Ho	0.682	0.774
	He	0.669	0.635
	F_{IS}	0.031	-0.226

Table 4.9. Continued.

SalJ81	N	22	31
	A	5	8
	Private A	1	4
	Allelic richness	6.89	6.85
	Size range	125-131	123-141
	Ho	0.545	0.613
	He	0.508	0.552
	F_{IS}	-0.169	-0.100
Omm1377	N	22	31
	A	5	9
	Private A	1	4
	Allelic richness	6.43	6.35
	Size range	259-265	251-267
	Ho	0.818	0.903
	He	0.698	0.777
	F_{IS}	-0.186	-0.161
Sfo334lav	N	22	31
	A	13	15
	Private A	4	6
	Allelic richness	11.70	11.70
	Size range	233-289	245-283
	Ho	0.727	0.806
	He	0.807	0.772
	F_{IS}	0.121	-0.040
SalO23	N	22	31
	A	15	15
	Private A	3	3
	Allelic richness	12.37	13.79
	Size range	213-279	209-253
	Ho	0.773	0.839*
	He	0.918	0.881
	F_{IS}	0.158	0.068
SalD30	N	22	31
	A	9	9
	Private A	4	4
	Allelic richness	6.94	8.19
	Size range	156-180	154-186
	Ho	0.591	0.710
	He	0.808	0.787
	F_{IS}	0.328	0.071
Total	Mean A	7.89	9.22
	Mean A. Rich ± SD	7.65 ± 2.81	7.95 ± 3.11
	Mean H_e ± SD	0.71 ± 0.17	0.69 ± 0.18
	Mean H_o ± SD	0.66 ± 0.22	0.71 ± 0.21
	Mean F_{IS}	0.081	-0.028

4.3.2.2. Population Structure

Pairwise comparisons of F_{ST} and Rho_{ST} excluding loci found to deviate from Hardy-Weinberg equilibrium gave negative values (-0.003 and -0.004 for F_{ST} and Rho_{ST} respectively) indicating no significant differentiation between the two sub groups distinguished by morphology. Pairwise comparisons over all loci gave positive values of both F_{ST} (0.017) and Rho_{ST} (0.002) but they remained non significant. The factorial correspondence analysis, on the other hand, indicated separation on the basis of allelic frequency between the two morphs at FCA 1 but not FCA 2 (Figure 4.3).

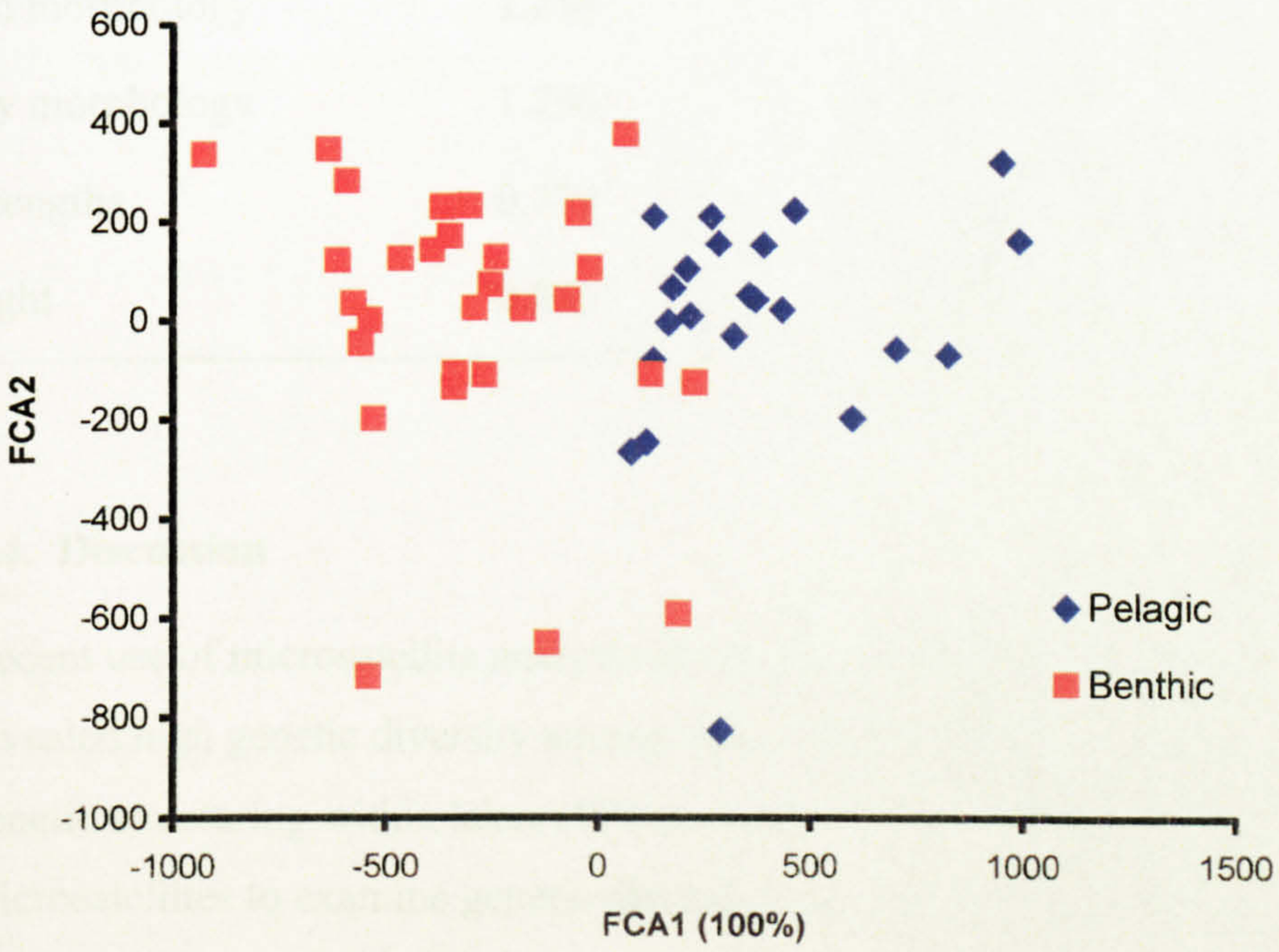


Figure 4.3. Genetic differentiation between Arctic charr morphotypes in Coniston Water, based on factorial correspondence analysis (FCA) of allelic frequency at six microsatellite loci.

4.3.2.3. Relationship between genetic variation and morphology in Coniston Water

The only set of predictor variables that had a significant relationship with genetic distances was head morphology (Table 4.10), which explains 15.5 % of the variation in genetic distances between individuals. This corresponds to the morphological results in Chapter Three showing that head morphology is the main factor distinguishing between pelagic and benthic morphs in Coniston Water.

Table 4.10. Results of sequential tests of predictor variables using the dbRDA multivariate *F*-statistic.

Set	*F*	*P*	% Variation
Head morphology	**1.250**	**0.05**	**15.5**
Body morphology	1.330	0.11	5.4
Fin lengths	0.732	0.86	3.0
Weight	0.856	0.63	1.7

4.4. Discussion

Recent use of microsatellite analysis of the genetic structure of Arctic charr has revealed high genetic diversity among lakes and allowed the detection of clear genetic structuring within lakes (Wilson et al. 2004). The present study has used microsatellites to examine genetic diversity and sub-structuring within lake systems in the Lake District, England.

4.4.1. Genetic differentiation between spawning populations in Windermere

There is significant genetic differentiation between the putative Windermere Arctic charr populations (Table 4.5., Figure 4.2.). Both autumn and spring spawning populations are separated to some degree by basin, with some indication of directional gene flow from the north basin to the south basin (Table 4.6). The largest degree of differentiation between sub populations however is between the autumn

and spring spawning populations in the south basin. This pattern is reflected in both the relatively high significant F_{ST} and Rho_{ST} values (Table 4.5) and in the FCA analysis. In the north basin, differentiation between the spawning populations is lower, as can be seen by the large overlap in FCA values, low F_{ST} and Rho_{ST} values and high migration rates from the spring spawning population to the autumn population (Figure 4.2; Tables 4.5 and 4.6). This increased gene flow from the spring spawning population to the autumn population within the north basin is surprising. A previous study, however, found a similar pattern of differentiation although gene flow was not estimated, and the results are only suggestive as based on just one allozyme locus. Partington and Mills (1988) found significant differences in allele frequencies at the esterase EST-1 locus between spawning populations (EST-1 115 allele frequency = 0.24 in autumn *cf.* 0.62 in spring population) within the south basin but not in the north basin. In fact the autumn spawning population in the south basin had a much lower frequency of EST-1 (115) allele than the other three putative populations, which had similar frequencies.

Lake morphometry, for example, lake depth, size or habitat availability, has previously been used to interpret patterns of population structure in Arctic charr (Riget *et al.* 2000). Riget et al. (2000) studied landlocked Arctic charr populations in a range of lakes in Greenland in order to assess any relationship between lake morphometry and population structure. The lakes ranged in size from small shallow lakes (0.01 km^2, maximum depth < 3.3m) to large deep lakes (43 km^2, maximum depth >200m) and the results showed a positive correlation between lake size and volume and the number of charr morphs present. The complexity of population structure was also related to water transparency and the presence or absence of three-spine sticklebacks.

Despite some genetic structuring based on spawning season and basin, as is seen by the inbreeding coefficients and factorial correspondence analysis, the dbRDA statistics of individual predictor variables suggest that, when basin is removed as a factor, the depth where individuals were caught on spawning grounds is a highly significant factor in explaining the observed genetic variation in both basins (Tables 4.7 and 4.8), and in the north basin this factor is more significant than spawning

season. Examination of the sampled spawning sites in Windermere revealed variation in spawning habitat, both within and between the two basins. Frost (1965) stated that the breeding habits of spawning populations of Arctic charr in both basins of Windermere differed in both season and depth of spawning sites. Those charr spawning in spring did so at depths of between 15 and 20 m whereas charr spawning in autumn spawned at depths of between 1 and 4 m. In this study, however, those sites where fish spawn in spring in the north basin appear to be a lot shallower than Frost (1965) suggested, as all the charr were caught at depths less than 10m at both autumn and spring spawning grounds (3m at the autumn sites *cf.* 10m at Holbeck spring site). The differences in depths between autumn and spring sites where the charr were caught in the south basin are a lot greater (4 m at Grass Holme *cf.* > 20m at South Rawlinsons Nab) (see Figure 4.1). On the other hand although the charr were caught at these depths, it is not certain that charr were not additionally spawning at greater depths. Further investigation of spawning sites would be required in order to ascertain the exact range of depths the charr spawn in these locations.

Differences in spawning depths between sympatric Arctic charr morphs occur in many lakes, but are often an effect of the distinct habitats utilised by the different morphs. For example, in lakes where a deep profundal morph occurs in sympatry with a littoral benthic morph, the deep profundal morph will reside and spawn in much deeper waters than the littoral morph (Westgaard et al. 2004). Differences in spawning depth have also been observed in other freshwater fish species, for example, whitefish (*Coregonus lavaretus*).

Næsje et al. (2004) recognised three forms of whitefish in Lake Femund, Norway; a deepwater morph which spawns at depths > 30 m, a shallow water morph which spawns at depths 2-5 m and a river morph which spawns in inlet rivers and in the mouth of the outlet river at depths < 3m. These morphs are genetically distinct, despite considerable overlap in spawning period, suggesting that spawning depth is an isolating factor. The availability of suitable spawning substrate may also affect the depth at which charr spawn. Arctic charr prefer stony substrates and do not

spawn in silted or weedy areas (Frost 1965). Increased siltation from land run-off or weed growth caused by eutrophication may reduce the availability of suitable substrate. For example, if siltation occurred at depth, where water movement is less strong, charr may move to more suitable shallower sites in order to avoid the suffocation of their eggs. Evidence of population decline due to the siltation of spawning grounds has been found for the Vendace (*Coregonus albula*) in Bassenthwaite (Winfield et al. 2004b). It is possible that similar processes are occurring in Windermere.

Theoretically, if spawning depth is a factor, the similarity in spawning depth observed in the north basin of Windermere could allow migrants to spawn on different sites of similar depth to their natal site, resulting in the lower genetic differentiation in the north basin compared to the south basin. This would be hindered by the variation in spawning season, unless the importance of temporal differences has become less significant, which could possibly occur due to climate changes affecting seasonal spawning cues.

The timing of spawning in salmonids is both genetic and plastic (Hendry 2001, Fleming and Reynolds 2003) and the movement of individuals between sub-populations in Windermere suggests a certain degree of plasticity in spawning behaviour. It would be selectively advantageous, for males especially, to be flexible as to spawning season and location in order to take advantage of any breeding opportunities.

The large directional gene flow of individuals from spring to autumn spawning populations may suggest that some individuals are spawning earlier in the year. The onset of spawning of freshwater fish occurs in response to environmental cues such as water temperature and light intensity (Winfield et al. 2004a). Studies of the effects of increases in water temperature on the spawning times of freshwater fish are limited to those species where long-term data sets are available. In Windermere this includes the European perch (*Perca fluviatilis*), which has advanced its spring spawning by 12 days over the period of 1946-2003, correlating strongly with

increasing water temperature in the first 15 weeks of the year (Winfield et al. 2004a). Other studies of this species have also found that populations have advanced spawning by up to one month elsewhere in Europe (Reist et al. 2006). Analysis of a phenological 44-year time series of the spawning of pike (*Esox lucius*) and bream (*Abramis brama*) in Estonia has shown that spawning has advanced by six days and eight days respectively in concordance with the onset of spring (Ahas 1999, Reist et al. 2006). Similar effects on the spawning of salmonid species, including Arctic charr, are likely to occur, especially given their sensitivity to temperature, although the long-term datasets required for this type of analysis are not available (Sparks et al. 2006).

The low migration rates between the north and south basins is further evidence that some individuals are not restricted to returning to the same spawning grounds year after year. This failure to home of a few individuals within populations is well documented amongst salmonids (Quinn and Dittman 1990, McDowall 2001). However, it is unclear whether individuals 'choose' to move to alternative spawning grounds, or whether certain individuals simply lack the ability to find their natal grounds. A failure to home may be selected for in order to maintain population diversity (McDowall 2001). However, the fact that the movement appears to be directional from the north basin to the south suggests that simply a failure to home is unlikely to be the cause, as this would most likely produce a random migration pattern. Instead, a choice to move to alternative spawning grounds may be the result of factors such as population density or site availability. Low female population density, for example, may cause males to move to alternative spawning grounds in order to find mating opportunities (Neville et al. 2006). Alternatively, high population densities or reductions in suitable spawning sites may cause competition for space forcing females to move to alternative sites. Although possible, the effects of these factors on natal homing patterns have not been tested experimentally.

In theory, in lakes where segregation between morphs persists and is associated with assortative mating based on temporal and/or spatial spawning segregation, the adaptive plastic responses of morphotype can become genetically assimilated. For

example, this has been seen in Lake Fjellfrøsvatn in Norway and Loch Rannoch in Scotland, where morphotype has been shown to have a inherited basis (Adams and Huntingford 2002b, Klemetsen et al. 2002). In the south basin of Windermere there are significant differences in morphology between the spawning populations (Chapter Three), and the dbRDA showed that some aspects of morphology, including body height, head depth and pectoral fin length were significant factors in explaining the variation in genetic distances, although they accounted for a much lower percentage than spawning depth or season. In the south basin, this correlation between morphotype and spawning behaviour suggests that both morphology and spawning behaviour are likely to be important factors in the significant genetic isolation of the populations that has been observed. In the north basin, on the other hand, there seems to be little association between morphotype and spawning season (Chapter Three), providing no evidence of a genetic basis to morphotype. This suggests that spawning behaviour, and, as can be seen by the dbRDA analysis, most importantly the depth at which an individual spawns, is the key factor in population sub-structuring in this basin.

This genetic sub-structuring in Windermere based on time and location of spawning suggests that to a certain degree individuals maintain the season and location in which they spawn from year to year. This is expected due to the natal homing instinct found in most salmonids (Hendry 2001, Fleming and Reynolds 2003). However it is important to note that the differentiation found between the sub-populations is weak, and migration rates between spawning sites is greater compared to that found in other sympatric populations of Arctic charr that differ in time and location of spawning (Table 4.11). This suggests that ongoing low levels of gene flow are maintaining diversity within the sub-populations, although why this occurs in Windermere to a greater extent than in other systems is unclear.

Table 4.11. Summarised information on the significant genetic differentiation among sympatric Arctic charr morphs from the literature.

Lake System	Morphs	Spawning segregation?	F_{ST} value	Reference
Fjellfrosvatn, N Norway	Littoral Profundal	Temporal (September vs March) Spatial	0.032	Westgaard et al. (2004)
Svínavatn, Iceland	Planktivore Benthivore		0.059	Gislason et al. (1999)
Vatnshlíoarvatn, Iceland	Small benthic Large bethic		0.058	Gislason et al. (1999)
Lake Galtaból, Iceland	Piscivore Benthivore		0.321	Gislason et al. (1999)

4.4.2. Genetic population structure within Coniston Water

The presence of discrete sympatric phenotypes in Arctic charr is often coupled with their genetic divergence (Gislason et al. 1999, Lu and Bernatchez 1999, Adams et al. 2006). Therefore genetic divergence is assumed to occur as a result of habitation in alternative habitats resulting in either temporal or spatial divergence of spawning or a combination of the two, or assortative mating based on phenotype.

In Coniston Water, negative pairwise values of both F_{ST} and Rho_{ST} in comparisons between the two morphs suggest little differentiation between them, despite high levels of phenotypic differentiation. However, low values of F_{ST} and Rho_{ST} can be a result of sampling or low statistical power. When pooled the two groups exhibit significant heterozygote deficits. Deviations from Hardy-Weinberg equilibrium in the form of heterozygote deficits can be interpreted as indirect evidence of population structuring within previously presumed randomly mating populations (Wahlund effect, Wahlund 1928), and could therefore be evidence of genetic differentiation within the population. They could also be evidence of null alleles but since the deficits are not consistently the same loci in both morphs this seems

unlikely. However, heterozygote deficits can also be caused by inbreeding, small population size and by disproportionate sampling (Castric et al. 2002). Although small population size and disproportionate sampling cannot be ruled out, any effects of inbreeding are unlikely given the low F_{IS} values.

Despite the non significant inbreeding coefficients, the factorial correspondence analysis does show separation between the morphs based on allele frequencies at FC1 (Figure 4.3), and the results of the distance based redundancy analysis indicate that differences in head morphology account for 15.5 % of variation in genetic distances within the population, suggesting that there is genetic differentiation between the two morphs. Other studies have successfully used this analysis to determine factors important in the genetic structuring of populations (e.g. Geffen et al. 2004). All the morphological traits assessed together accounted for 25.6 % of the genetic variation within the population. Other possible environmental factors that could explain the patterns in genetic variation could include diet, temperature, habitat or depth at which they were caught. It is unlikely that factors such as temperature or depth caught would be of use when studying such mobile animals, as the habitat where an individual is caught does not necessarily represent where it spends the majority of its time. However, one alternative factor that may cause genetic structuring within a population is homing to discrete spawning sites (Adams et al. 2006) and, although all individuals in Coniston Water are thought to spawn during spring, little is know about the localities of spawning sites and it is possible that spawning occurs at more than one location or at different depths.

There are commonly two possible scenarios that cause the presence of sympatric polymorphisms in lake-dwelling freshwater fish (e.g. Næsje et al. 2004). In the first scenario, morphotypes have an allopatric origin and independently colonise the lake system and thus occupy independent niches. In the second scenario, a monomorphic ancestor colonises the lake and radiates into the available niches as a result of phenotypic or behavioural plasticity. Phenotypic variation based on phenotypic plasticity, where morphology is a plastic response to alternative environments during development, is common in Arctic charr (Fraser et al. 1998, Adams and Huntingford

2004, Andersson et al. 2005), as well as many other freshwater fish species (Hegrenes 2001). By this mechanism, alternative morphologies are produced within a population by the differential expression of genes (Meyer 1987), usually in response to variable availability of different prey types during development. In the case of phenotypic plasticity no genetic divergence would be expected unless the phenotypic traits are associated with positive assortative mating (Rice and Hostert 1993). On the other hand, behavioural plasticity involving variation in spawning behaviour, for example, spawning in different locations or seasons, could also result in reproductive isolation and genetic divergence.

The shallow sub-structuring observed in Coniston Water suggests that the genetic segregation of morphs is very recent and incomplete. This rules out independent colonisation and suggests instead that morphs diverged in sympatry after postglacial colonisation of a single morph. The correlation between morphotype and genetic variation suggests that the processes of reproductive isolation acting on the populations are to some extent, related to morphology and the use of divergent niches. This however could either have a plastic or genetic basis, which cannot be determined without laboratory breeding experiments.

4.4.3. The role of phenotypic plasticity in the genetic divergence of Arctic charr populations in sympatry

Ghalambor et al. (2007) outlined possible mechanisms whereby phenotypic plasticity aids the adaptive evolution of populations when colonising new environments. The most common mechanism is that phenotypic plasticity produces a mean phenotype below the optimum in the new environment. The population will then be subject to directional selection allowing the population to become adapted to the new environment. Multiple morphs can occur in the same way in response to multiple niches and diversifying selection acts to maintain multiple phenotypes within that selective environment, for example, the two morphs in Coniston Water and two spawning seasons in Windermere.

In this scenario, adaptation to different niches will eventually cause the plastic traits to be genetically assimilated (Waddington 1961); they gradually become canalised and develop in the absence of the triggering environmental stimulus (reviewed in (Pigluicci and Murren 2003, West-Eberhard 2003). This has been suggested in examples of Arctic charr systems where different morphotypes occurring sympatrically have been shown to have a genetic basis (Adams and Huntingford 2002b, Klemetsen et al. 2002). Further examples include the genetic assimilation of environmentally induced changes in leg length in Caribbean *Anolis* lizards, which is thought to have occurred during their adaptive radiation (Losos et al. 2000). Although it is uncertain whether the morphotypes in Coniston Water have a genetic basis, their genetic divergence provides evidence that conditions required for the genetic assimilation of morphological traits are present. This process may also help explain the presence of the two morphs despite the apparent absence of extensive dietary segregation (see Chapter Two), as once genetically assimilated the morphs can occur in the absence of environmental stimulus. On the other hand, the apparent absence of dietary segregation may also be due to sampling, for example, the time of year in which they were caught. Polymorphisms with a complete genetic basis can be maintained in equilibrium within one population by density-dependent balancing selection, evidence of which has been found for two reproductive strategists of the pumpkinseed sunfish, *Lepomis gibbosus* (Rios-Cardenas and Webster 2008).

Genetic assimilation or canalisation theoretically involves a reduction in plasticity. This decreases the ability of producing future adaptive phenotypes to buffer environmental change and thus reduces the fitness of the population (West-Eberhard 2003). In the case of Arctic charr and fishes in general most examples show potential for developmental plasticity even when traits are genetically assimilated. For example, traits that have been shown to be genetically assimilated in breeding experiments are also plastic when organisms are reared under variable environmental conditions (Alexander and Adams 2004). West-Eberhard (2003) therefore describes the less restrictive term 'genetic accommodation', which is genetic assimilation that does not necessarily lead to a loss in plasticity. In fact, she suggests that plasticity itself is under directional selection and under certain selective environments will

increase within a population. According to this theory, the plasticity is still environmentally induced but there must be an underlying genetic propensity for the capability to express alternative phenotypes.

Another possible mechanism is that phenotypic plasticity produces multiple phenotypes that are optimal in the new environment and can occupy all available niches. In this case no genetic differentiation will occur (Price et al. 2003). This type of adaptive plasticity has been documented in a variety of freshwater fish systems including pumpkinseed sunfish (Mittelbach et al. 1999) and Arctic charr (Hindar and Jonsson 1993). Mittelbach et al. (1999) reared offspring from two trophic morphs of pumpkinseed sunfish from lakes in Michigan, USA, in common environments both in the laboratory and in ponds and found that the offspring exhibited little difference in jaw morphology. Also by feeding offspring from both morphs different sized snails, variation in jaw morphology could be induced, suggesting that the morphological variation in these populations of pumpkinseed sunfish is solely due to phenotypic plasticity. Further evidence of this scenario in Arctic charr can be seen in populations from Norway where breeding experiments have shown morphology to be entirely environmentally regulated (Hindar and Johnson 1993).

4.4.4. Comparison of genetic divergence in Windermere and Coniston Water

The reasons for the differing degrees of genetic variation between charr morphs within different lakes is unclear, but likely to be related to the magnitude and persistence of ecological segregation of morphs as well as the age of the systems (Skulason et al. 1999). The relative age of the system can be ruled out as a factor explaining the variation in patterns of genetic divergence between Windermere and Coniston Water, as they are thought to be approximately the same age (Evans et al. 2005). Gislason et al. (1999) studied five lakes with sympatric morphs of Arctic charr and found a significant correlation between the degree of morphological and genetic divergence indicating a coupling of these two processes. However a comparison of Windermere and Coniston Water does not fit with this correlation. Coniston Water has the greater magnitude of ecological segregation with low

reproductive isolation between morphs, whereas Windermere populations have less defined phenotypic variation but are separated by temporal and spatial spawning variation. Different environments and selection pressures within the two lakes since their colonisation by Arctic charr appear to have acted to shape the populations within each lake. There can also be significant correlations between the magnitude of ecological divergence and both the presence or absence of potential competitors, and with the diversity and density of other species present (reviewed in Schluter 2000), where divergence tends to be greatest in depauperate environments. Windermere presently hosts a diverse fish community and the potential for both interspecific competition for resources and predation is likely to be high, which may well affect processes of divergence. It does, however, have a diverse range of littoral habitats and suitable spawning sites are likely to be common. Habitat diversity could be a key factor in determining the degree of both phenotypic and genetic divergence of Arctic charr within these lakes. In Windermere, this provides a situation where generalist feeders that can consume the variety of available prey, as described in Chapter Two, are selected for in order to minimise competition with other species and differentiation based on variation of spawning grounds occurs.

Chapter 5 : Genetic differentiation between six English Lakes, Cumbria, UK

5.1. Introduction

5.1.1. Genetic diversity

During the last glacial maximum, ice sheets covered much of the British Isles until the onset of the present interglacial period ~18,000 BP. The Lake District is a radial drainage system with all of the glacially excavated valleys retreating upland to a central highpoint. During the Pleistocene glaciations, individual ice sheets extending from this central point would have covered each river basin (Evans et al. 2005). The retreat of these ice sheets would have opened up habitat for the colonisation of freshwater pioneer species, including Arctic charr, from coastal refugia (Wilson et al. 2004). These founding populations were likely to be small and the resultant loss of alleles may have caused reductions in genetic diversity (Hewitt 1996). DNA studies have shown patterns of reduced diversity in other postglacial expansions of freshwater fishes. For example, lake whitefish in North American lakes occupy most of the area that was previously covered by the Laurentide ice sheet and the putative refugial populations have higher diversity than those that have undergone extensive colonisation (Bernatchez and Dodson 1991).

5.1.2. Population genetic structure

Population genetic structure of freshwater fishes is expected to reflect historical patterns of dispersal and isolation, as well as contemporary dispersal and gene flow (Wilson et al. 2004). The ranges and population genetic structure of northern freshwater fishes have been heavily influenced by repeated glaciations during the Pleistocene epoch (Bernatchez and Wilson 1998). This is especially the case for Arctic charr, a pioneer species that probably repeatedly colonised habitats created by retreating and advancing ice margins (Johnson 1980). Glacial cover persisted over most of the current distribution of Arctic charr, until the end of the Pleistocene (~18,000 years ago); therefore their last range recolonisation is relatively recent. This is supported by several studies of the fast evolving mitochondrial DNA (mtDNA) control region sequence variation in Arctic charr (e.g. Hartley et al. 1992,

Brunner et al. 2001), which have shown little genetic differentiation between European regions studied including Scotland, Ireland and Alpine regions. This has led to the conclusion that despite high levels of phenotypic variability, all populations in northern Europe are derived from a single Atlantic lineage, which diverged from other charr lineages in the early to mid-Pleistocene (Brunner et al. 2001). Wilson et al. (2004) found results consistent with those of Brunner et al. (2001) using microsatellite loci, which have the potential to detect more recent phylogeographical patterns not resolved by using mtDNA. These results support the theory of rapid recolonisation from a single refugium after the last deglaciation. The recent genetic differentiation and phenotypic variability of Arctic charr between lakes throughout Europe described in Chapter Four suggests, however, that subsequent geneflow has been limited in certain areas; probably by receding water levels or possibly by a rise in temperature limiting anadromous migration (Wilson et al. 2004).

Within the Lake District, genetic structure of Arctic charr has been assessed using isozyme loci, serum transferrin and serum esterase, in one case (Child 1984) and skeletal muscle malate dehydrogenase and serum esterase, in another case (Partington and Mills 1988). Child (1984) found significant differences in allele frequencies at both loci in the Coniston Water population compared to Windermere and Ennerdale. Child (1984) concluded that the Coniston Water population might have undergone a historical bottleneck, which coincided with historical reports of a severe population decline in the late 1800s. Partington and Mills (1988) however found no further evidence of this.

5.1.3. Conservation and Management

Recent impetus for resolving the population genetic structure of Arctic charr on small spatial scales has come from a recognised need to implement conservation and management strategies throughout its range (Maitland 1995). Arctic charr is recognised as a species of high biodiversity conservation value as well as being one of few lake fishes in Britain to be exploited on a semi-commercial basis, and is therefore of considerable economic importance (Maitland 1995). The species is also

very sensitive to anthropogenic pollution and local population extinctions in Scotland have been attributed to acidification and eutrophication of water bodies (Maitland 1992, Maitland et al. 2007). In England extinctions have occurred in Goat's Water, Ullswater, Loweswater and Rydal Water (Maitland et al. 2007) leaving eight extant populations in Windermere, Buttermere, Thirlmere, Coniston Water, Ennerdale, Wast Water, Haweswater and Crummock Water, although there are concerns over future stocks in Coniston Water (Winfield et al. 2004c), Ennerdale Water (Maitland et al. 2007) and Windermere (Winfield and Durie 2004).

Concern for the remaining Arctic charr populations in Cumbria has led to long-term population studies within several lakes (e.g. Winfield et al. 2004). Particular attention has been paid to the populations within Windermere, which has recently suffered impacts from eutrophication, surface temperature rises and invasive species (see Chapter Two for details). A controversial splitting of the charr stocks in Cumbria into two independent species based on spawning behaviour and some limited morphological data alone is described by Kottelat and Freyhof (2007). The autumn spawning 'species', and the spring spawning 'species', both described in Chapter Three, co-occur only in Windermere whereas the autumn spawning species also occurs in Ennerdale (Frost 1965). All the other charr populations in the Lake District are described as the spring spawning species. The resolution of population genetic structure is therefore important in the management context, for the determination of genetically differentiated units for conservation (Moritz 1994). Although there has been no known stocking in the Lake District, lakes in Scotland and the Alps have been stocked for fisheries purposes, and information on population genetic structure is useful for informing any future stocking practices that preserve genetic integrity.

5.1.4. Chapter Aims

The objective of this chapter was to examine the genetic diversity and structure of populations of Arctic charr sampled from lakes in the Lake District National Park, Cumbria, UK, using both mtDNA and microsatellite DNA markers, in order to test the following hypotheses:

1. Significant genetic differentiation between Arctic charr populations of the lakes has occurred since their colonisation
2. Arctic charr in all the lakes are derived from a single ancestral lineage
3. Arctic charr have undergone a recent demographic expansion since the colonisation of the lakes from glacial refugia.

Although the population genetic structure of Arctic charr in some regions of the British Isles, particularly in Ireland and Scotland, is well understood (Wilson et al. 2004; Adams et al. 2006), no previous study has attempted to assess the population genetic structure of charr in the English lakes. The study of mtDNA markers allows comparisons with earlier work that assessed the variation of the mtDNA control region in Arctic charr populations throughout its range, including those in Canada, Greenland, Iceland and Northern Europe (Brunner et al 2001; Wilson et al. 2004). Microsatellite markers are also used as they are expected to provide greater resolution than other marker types, and are thus able to resolve the shallow inter-population relationships expected, given the comparatively short time since their colonisation.

5.2. Methods

5.2.1. Samples obtained

Details of the sampling methods and the samples obtained for genetic analysis from Windermere are outlined in Chapters Four and those from Coniston Water and Wastwater are provided in Chapters Three and Four. Further samples were collected by CEH Lancaster using the same sampling methods from Ennerdale Water, Buttermere, and Haweswater (Figure 5.1). Samples from Loch Girlsta, Scotland, were collected by the CEH Lancaster as part of an assessment of fish communities in Scottish standing waters for the Scottish National Heritage (See Winfield et al. 2006 for details) and were used as an outgroup for comparison. Total sample sizes are given in Table 5.1.

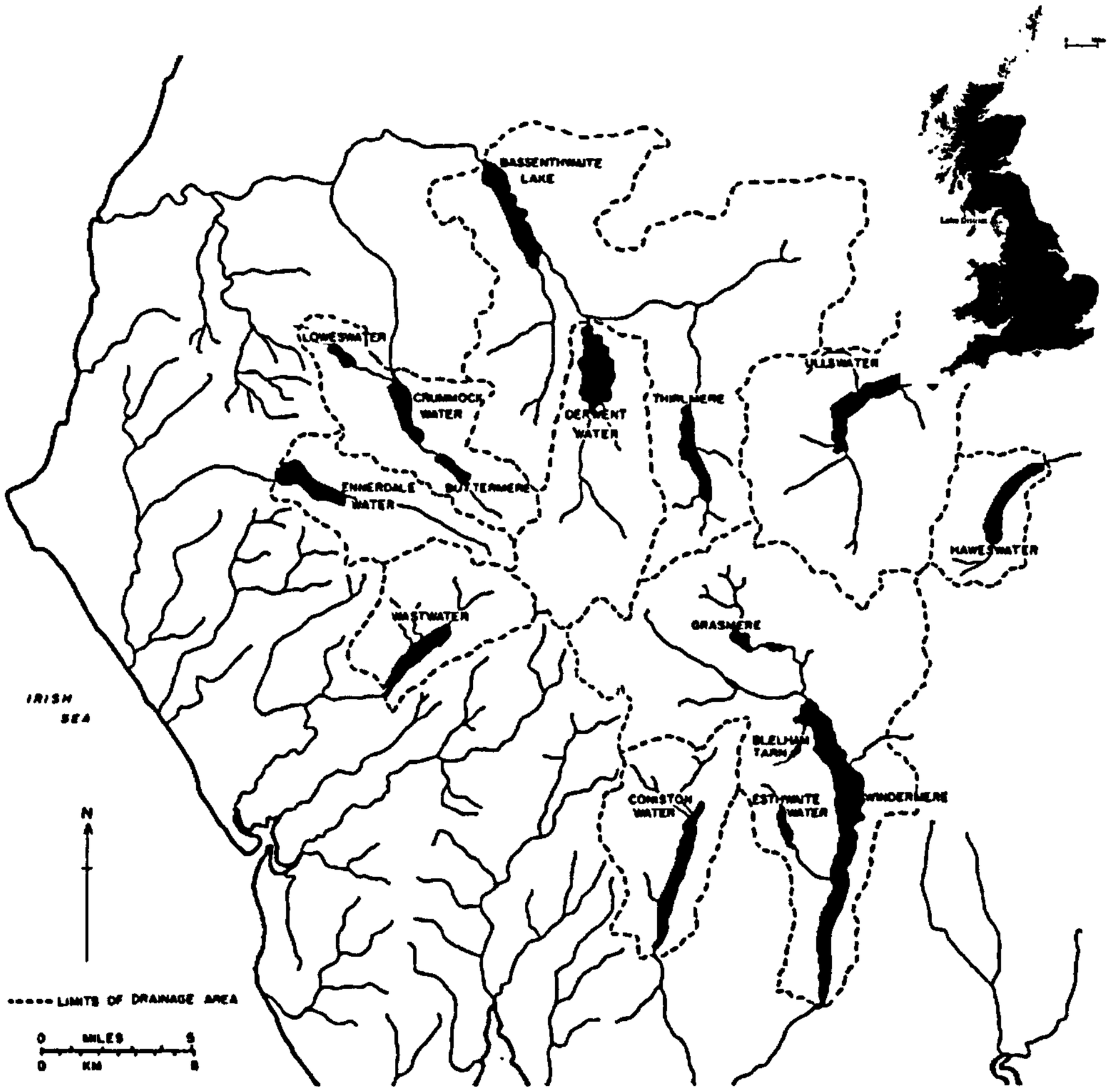


Figure 5.1. Map showing the location of the lakes sampled within the Lake District, UK

Table 5.1. Number of samples of Arctic charr from Lake District lakes used for microsatellite and mitochondrial analysis

Lake	Microsatellite sample number	Mitochondrial sample number
Windermere	163	20
Coniston Water	77	8
Buttermere	12	10
Ennerdale	7	7
Haweswater	7	7
Wast Water	30	10
Loch Girlsta	30	4

5.2.2. Genetic Analyses and determination of population structure

5.2.2.1. Genetic methods

Details of the techniques used to extract and isolate DNA and the amplification and interpretation of the microsatellite data has been provided in Chapter Four. The microsatellites used are given in Table 5.2.

500 base pair fragments of the mitochondrial DNA control region were sequenced for a subset of samples from each location (Table 5.1), following polymerase chain reaction (PCR) using the following primers;

Forward 5′-3′: TACCCCTTAACTCCCAAAG

Reverse 5′-3′: GACACCATTAACCAGATG

These species specific primers were designed within the conserved regions either side of the 500 base pair variable site of several aligned sequences of Arctic charr mitochondrial control region from Genbank (Benson et al. 2007). Purification of the PCR product followed the instructions from a commercial kit (QIAquick, QIAGEN, Inc., Valencia, CA). DNA sequences were then generated using commercial services (MACROGEN Inc.). All samples were sequenced in the forward direction and subsequently aligned using the program Clustal X (Larkin et al. 2004).

Table 5.2. Microsatellite loci, their primer sequences and annealing temperatures (T^a °C). In each case the forward primer was fluorescently labelled with the dye indicated

Locus	Primer Sequence (5'–3')	Reference	T^a (°C)	Allele Size (bp)	Repeat Motif	Dye
Sfo334 lav	GGA TTA ACA GAA GGT TAC TG CTT CGT ATT CTT CAT TGT GC	Perry et al (2005)	52	216-296	$(TG)_{58}G_4(TG)_{27}(CGTG)_8$	FAM
Smm 17	AAG GAT GGT GAG GAC AAT ACA ACC TTG AGA AAT CTA TAT GTG GTC TA	Crane et al (2004)	58	107-131	$(CA)_{29}$	FAM
Sal O23	TCT GAA TGC AGC CCC ACA G TTC AAG CCA AAG GAC ACA TGG	McGowan et al (2004)	60	210	$(TG)_{60}$	HEX
Ssa 85	AGG TGG GTC CTC CAA GCT AC ACC CGC TCC TCA CTT AAT C	O'Reilly et al (1996)	60	161-211	$(GT)_{14}$	NED
Smm 10	AAA ATG TCT CCC CTC CCT CTC TCC CTA ACA TAA CAA GTT TTC ATC CT	Crane et al (2004)	55	150-170	$(TCCA)_{16}$	FAM
Sal J 81	CAG CAT AAT CAC TCC CGC GAA AGC TAC CTT GCG TGC	McGowan et al (2004)	52	125-149	$(GT)_{33}$	HEX
Sal D 30	TTT GTT GTT ATG ACT CTG CG CAA GCA GAA TCG TTT GGT C	McGowan et al (2004)	55	158-178	$(AC)_{52}$	FAM
Omm1302	AGC CAG CCA ATT AAT ACC CTG TTC TGT GTG GCC TAA ACC TT	Palti et al (2002)	58	184-212	CATC/CT	HEX
Omm1377	TGT GTC TCT CAA TGC GAC CTG GGC AAA ACT CCA CGG ACT GTA	Rodiguez et al (2003)	58	258-268	(CA)* imperfect repeat	FAM
Mst 85	GGA AGG AAG GGA GAA AGG T GGA AAA TCA ATA CTA ACA A	Presa and Guoymard (1996)	52	202-238	$(CT)_{22}$	NED

5.2.2.2. Statistical analysis

Before proceeding with analysis, genotyping errors were investigated using Micro-checker as described in Chapter Four. Polymorphism was estimated as the number of alleles per locus, number of private alleles per putative population, allelic richness, observed and expected heterozygosity, calculated for each locus in each lake using GENEPOP version 3.3 (Raymond and Rousset 2001). Deviation from Hardy-Weinburg equilibrium was tested using a method analogous to Fisher's exact test using a modified version of the Markov-chain method (Guo and Thompson 1992), implemented in ARLEQUIN 2.000 (Schneider et al. 2000).

A global value of IAM estimate of F_{ST} (Weir and Cockerham 1984)) was calculated using FSTAT version 2.9.3 (Goudet 2001) for all lakes. Confidence intervals for multilocus estimates were obtained by bootstrapping across loci, and region-specific estimates of F_{ST} were compared by randomising lakes across regions (Goudet 2001). Rho_{ST} (Rousset 1996) was calculated in the same way using RSTAT . Genetic differentiation between specific lake pairs was also assessed using pairwise estimates of F_{ST} and Rho_{ST}, as described in Chapter Four.

In addition to F_{ST} and Rho_{ST}, several other methods were used to assess the genetic relationships among lakes. A factorial correspondence analysis (FCA), described in Chapter Four, was performed using the program Genetix 4.0 (Belkhir 1999). The use of FCA to analyse genetic data has been described by (She et al. 1987) and is used in order to give a visual representation of individuals clustering on the basis of their relative allele frequencies. Multidimensional scaling (MDS), implemented in XLSTAT was also used to give a visual representation of the similarity between individuals represented by genetic distance. The genetic distance matrix used was the Infinite Allele Model (IAM) based chord distance (D_{CE}) (Cavalli-Sforza and Edwards 1967) calculated using the program 'Populations' version 1.2.24 (Langella 2001). Both FCA and MDS were used to examine the genetic structure among individuals without *a priori* assignment to lake populations.

Genetic relationships among populations were also examined by constructing an unrooted tree, using the suite of programs contained in PHYLIP version 3.5c (Felsenstein 1993). Tree topography was determined using chord distance (D_{CE}) (Cavalli-Sforza and Edwards 1967) and a neighbour-joining algorithm, with confidence determined by bootstrapping across loci with 1000 replicates.

Mitochondrial DNA polymorphism was estimated as haplotypic diversity (h; Nei and Tajima 1981, Nei 1987), nucleotide diversity (π, Nei 1987), and as net percentage sequence divergence between haplotypes. A neighbour-joining tree was constructed with MEGA version 3.1 (Kumar et al. 2004) to assess the phylogenetic relationships among populations. Additionally, parsimony and maximum-likelihood analyses were conducted with PAUP version 4.0 (Swofford 2000). The significance of the trees generated was tested by resampling 1000 times to obtain bootstrap *P*-values (Felsenstein 1985).

Two tests for neutrality were performed in ARLEQUIN 2.000 (Schneider et al. 2000); Tajima's 'D' (Tajima 1989) and Fu's 'Fs' (Fu 1997). Tajima's test of neutrality (Tajima 1989) compares the average number of pairwise nucleotide differences (*k*) between haplotypes in a sample (M) expected from the number of segregating sites (*K*). This determines whether the sequences are evolving randomly as expected under neutral theory or if they are affected by alternative mechanisms such as selection, gene flow, demographic expansion or decline. Therefore, rejection of neutrality may suggest that the population has undergone demographic expansion (Tajima 1989).

Mismatch distribution analyses were used to evaluate possible events of population expansion (Rogers and Harpending 1992) using DnaSP 4.50 (Rozas et al. 2003). This analysis also calculates an estimate of *Tau* which can be used to estimate divergence time (*t*) using the equation $Tau = 2\mu t$ where μ is the mutation rate. This analysis was carried out twice; once using mtDNA sequence data from the Lake District only and again with additional mtDNA sequence data from the 'Atlantic' lineage described by Brunner et al. (2001) obtained from Genbank (Table 5.3).

Table 5.3. Sample sizes and locations of haplotypes from the Atlantic lineage of Arctic charr taken from Brunner et al (2001).

Haplotype	Sample Locations						
	Lake District	Alpine Region	Norway	Finland	Iceland	Greenland	Arctic Canada
H_1 (ATL1)	39	22	6			1	
H_3 (ATL 18)							2
H_4 (ATL17)							1
H_5 (ATL14)		2					
H_6 (ATL13)			2				
H_7 (ATL12)			2				
H_8 (ATL11)						1	
H_9 (ATL 10)						1	
H_10 (ATL9)						2	
H_11 (ATL7)		2					
H_12 (ATL 4)						3	
H_13 (ATL 3)	2						

Table 5.4. Sample sizes and locations of haplotypes from the Arctic lineage of Arctic charr taken from Brunner et al. (2001).

Haplotype	Sample locations			
	Greenland	Eastern Siberia	Alaska	Arctic Canada
ARC1		2		
ARC2		2		
ARC3		1		
ARC4	2			4
ARC5			2	
ARC6				1
ARC7				3
ARC8				2
ARC9				4
ARC10				2
ARC11				2
ARC12				2
ARC13				2
ARC14				2
ARC15	1			
ARC16	1			
ARC17			1	
ARC18				2

Pairwise divergence times between lakes were estimated with a coalescent-based approach using the program MDIV (Nielsen and Wakely 2001). MDIV simultaneously generates estimates of theta ($\theta = 2N_{ef}\mu$) where N_{ef} is the effective population size and μ is the mutation rate, the migration rate ($M = 2N_{ef}m$), the time of population divergence ($T = t/N_{ef}$) and expected time to the most recent common ancestor (TMRCA = $t\mu$) from sequence data. Comparisons within the Lake District were restricted to the following (due to small sample sizes at the other lakes): Windermere vs Coniston Water, Windermere vs Wastwater and Coniston Water vs Wastwater. The Lake District lake samples were also grouped and run against sequences from the 'Atlantic' lineage (Table 5.3) and the 'Arctic' lineage (Table 5.4) from Brunner et al. (2001). Sequences were chosen from these lineages as the 'Atlantic' is considered to be the most recent lineage whereas the 'Arctic' is considered the most ancient (Brunner et al (2001). Tables 5.3 and 5.4 list the sampling locations where haplotypes from each lineage were found. The program was run under the finite sites model, with 5×10^6 cycles with a 10 % burn-in period. Likelihood values for θ, M and T were calculated and the values with the highest posterior probability were accepted as the best estimates. Values for t and TMRCA were then calculated using an estimated mutation rate of between 5×10^{-9} and 1×10^{-7} substitutions/site/year, which corresponds to a percentage sequence divergence rate of 5-100%. These rates were then rescaled by the sequence length (460 nucleotides) and generation time of 3 to give a value of $\mu = 7.5 \times 10^{-6}$ and $\mu = 1.5 \times 10^{-4}$.

5.3. Results

5.3.1. Microsatellite analysis

Over all lakes the mean number of alleles per locus ranged from 4.3 to 16.4, with a mean over all loci and all lakes of 9. Observed heterozygosity also varied across loci assayed, with mean H_O over all lakes ranging from 0.53 to 0.80, with a mean over all loci and over all lakes of 0.65 (Table 5.5). Detailed diversity measures per locus are provided in Appendix I.

Table 5.5. Mean values (over all loci) for number of alleles (A), allelic richness (R), observed (H_O) and expected (H_E) heterozygosity for each lake sampled. *P* values are indicated for multilocus Hardy-Weinberg equilibrium tested against the alternative hypothesis of heterozygote deficit.

		Mean over all loci				
Lake	N	A	R	H_E	H_O	P
Windermere	170	18.2	4.0	0.81	0.78	0.136
Coniston Water	77	12.2	3.4	0.69	0.64	0.059
Buttermere	12	5.3	2.9	0.62	0.54	0.439
Ennerdale	7	5.3	3.6	0.74	0.60	0.368
Haweswater	7	6.0	3.8	0.76	0.71	0.390
Wast Water	30	9.9	3.8	0.74	0.70	0.415
Loch Girlsta	30	6.1	4.6	0.64	0.70	0.249

5.3.1.1. Genotyping errors and tests for Hardy-Weinberg equilibrium

Homozygote excess as a result of large allele dropout or null alleles was found for the locus Sfo334 in the samples from Windermere (estimated null allele frequency = 0.17) and Wastwater (estimated frequency = 0.13), for the locus SalJ81 (estimated frequency = 0.18) in Wastwater and for the locus Smm10 and SalD30 in Coniston Water (estimated frequencies = 0.09 and 0.07, respectively). The factorial correspondence analysis was carried out using nine loci, dropping a single locus at a time, to assess whether any of these loci would bias the results (Appendix Two). Sfo334 was the only locus that changed the pattern of differentiation. Therefore it was removed from further analysis leaving nine loci. No significant multilocus heterozygote deficiencies compared against Hardy-Weinberg expectations were found for any population (Table 5.5). However, heterozygote deficiency at some individual loci was observed in all populations (Appendix One). Further tests of sub-populations in Windermere and Coniston Water, however, found that significant heterozygote deficits were then confined to Smm17 and SalO23 in Coniston Water only and that their omission did not alter the pattern of within-lake genetic structure (Chapter Four). Heterozygote deficiency compared to Hardy-Weinberg expectations

were also found for locus SalO23 and Omm1377 in the Buttermere population, for Smm17 in the Ennerdale population, for SalO23 and SalD30 in the Wast Water population and for SalO23 in Loch Girlsta (Appendix One).

5.3.1.2. Population genetic structure

The sample sizes from Buttermere, Ennerdale and Haweswater were too low to be included in the analysis of genetic structure using inbreeding coefficients and therefore values are given for the remaining lakes only. The estimate of global F_{ST} (standard error in parenthesis) over all lakes from the Lake District region was F_{ST} = 0.157 (0.027). Within the Lake District lakes the pairwise values of F_{ST} had a mean over all pairs of 0.160 and a maximum value of 0.209 between Coniston Water and Wastwater, confirming genetic differentiation between the lakes (Table 5.6). Estimates of F_{ST} were highly significant in all cases and were all of a similar magnitude. Pairwise estimates of Rho_{ST} were also of similar magnitude and were significant in all cases. Pairwise estimates of both F_{ST} and Rho_{ST} were highest between Loch Girlsta and all three Lake District lakes.

Table 5.6. Pairwise values of F_{ST} (below diagonal) and Rho_{ST} (above the diagonal) using nine microsatellite loci minus Sfo334. Values with all loci exhibiting null alleles are given in parenthesis (* denotes values are significantly greater than zero at 0.05 significance).

	Windermere	Coniston Water	Wastwater	Loch Girlsta
Windermere		0.225* (0.153*)	0.117* (0.123*)	0.645* (0.588*)
Coniston Water	0.145* (0.117*)		0.281* (0.154*)	0.762* (0.673*)
Wastwater	0.153* (0.155*)	0.216* (0.209*)		0.694* (0.684)
Loch Girlsta	0.199* (0.205*)	0.318* (0.240*)	0.280* (0.266*)	

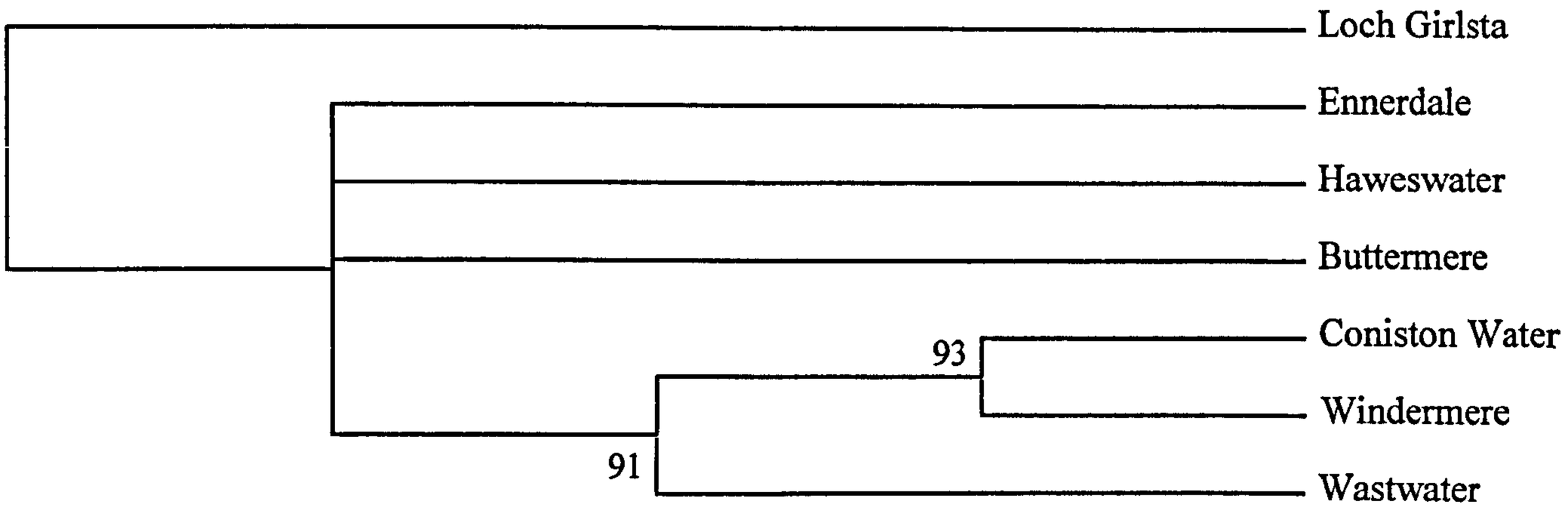
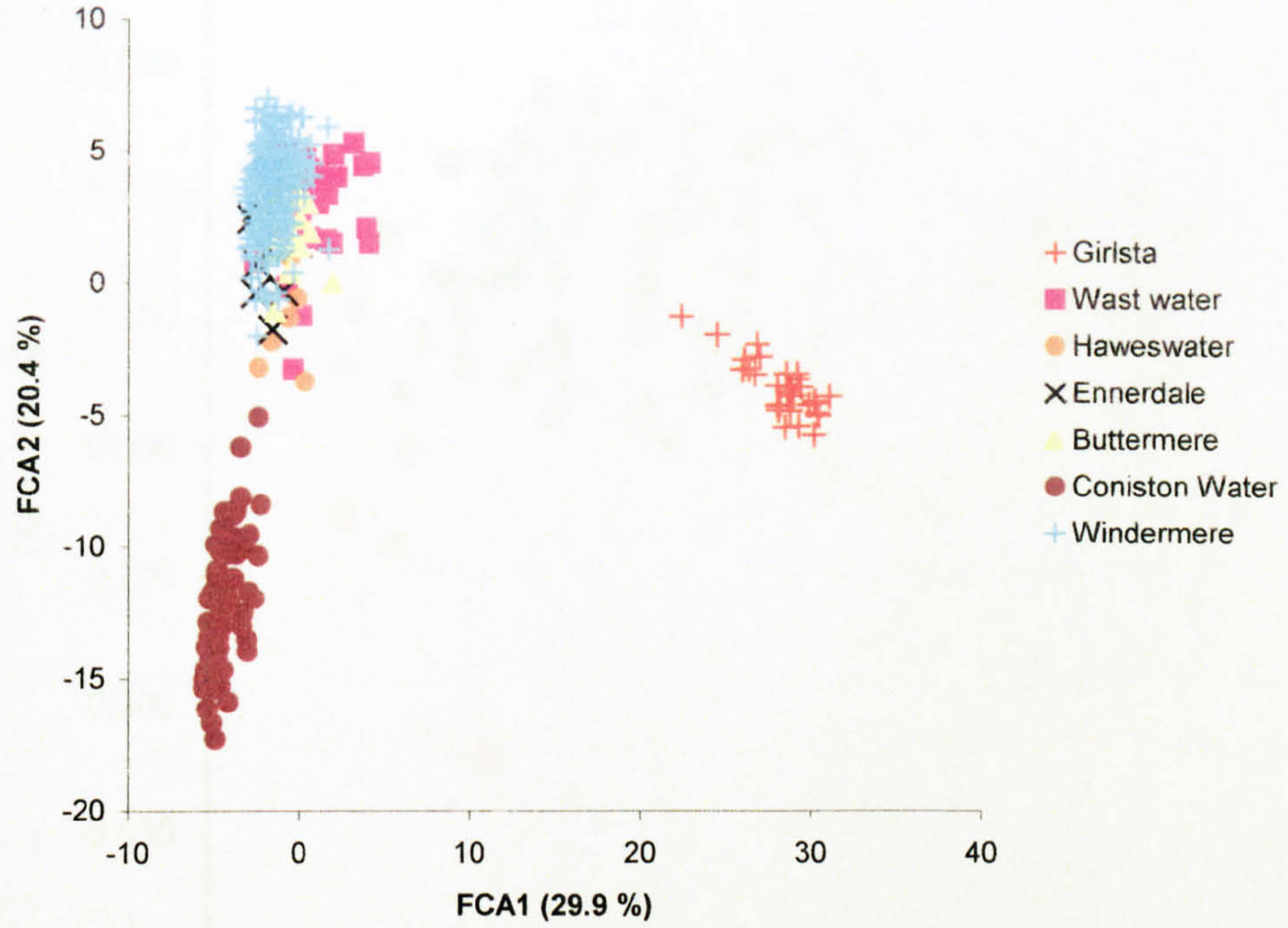


Figure 5.2. Neighbour-joining consensus tree of Arctic charr populations constructed using D_c genetic distances based on 9 microsatellite loci. Tree is rooted to Loch Girlsta as an out-group. Bootstrap values out of 1000 are given as percentages.

The neighbour-joining tree, rooted to Loch Girlsta, showed the same pattern with 6 (excluding those with evidence of nulls) and 9 loci, so the latter is presented (Figure 5.2). The tree showed strong branch support for the relationship between Coniston Water, Windermere and Wastwater, with Coniston Water and Windermere having the stronger relationship. There was no branch support for the relationships involving Ennerdale, Buttermere and Haweswater, however this may be due to the low sample sizes from these lakes. As indicated above, the Factorial correspondence analysis (FCA) was repeated 10 times, in each case omitting one locus. All patterns showed the same relationship among clusters (Appendix Two), apart from the one that omitted locus Sfo334 (Figure 5.3). Both analyses (with and without Sfo334) showed the Lake District lakes separated from Loch Girlsta at FCA 1, which represented the greatest proportion of variation within the sample. The omission of Sfo334 primarily affects the position of Wastwater samples, moving them from close association with Windermere to association with Coniston Water, consistent with the

relatively substantial proportion of nulls estimated for this locus at Wastwater and Windermere. The multidimensional scaling (MDS) based on nine microsatellite loci was generally consistent with the FCA, and again showed that despite variation between individuals and some degree of overlap, individuals clustered together by lake (Figure 5.4).

a)



b)

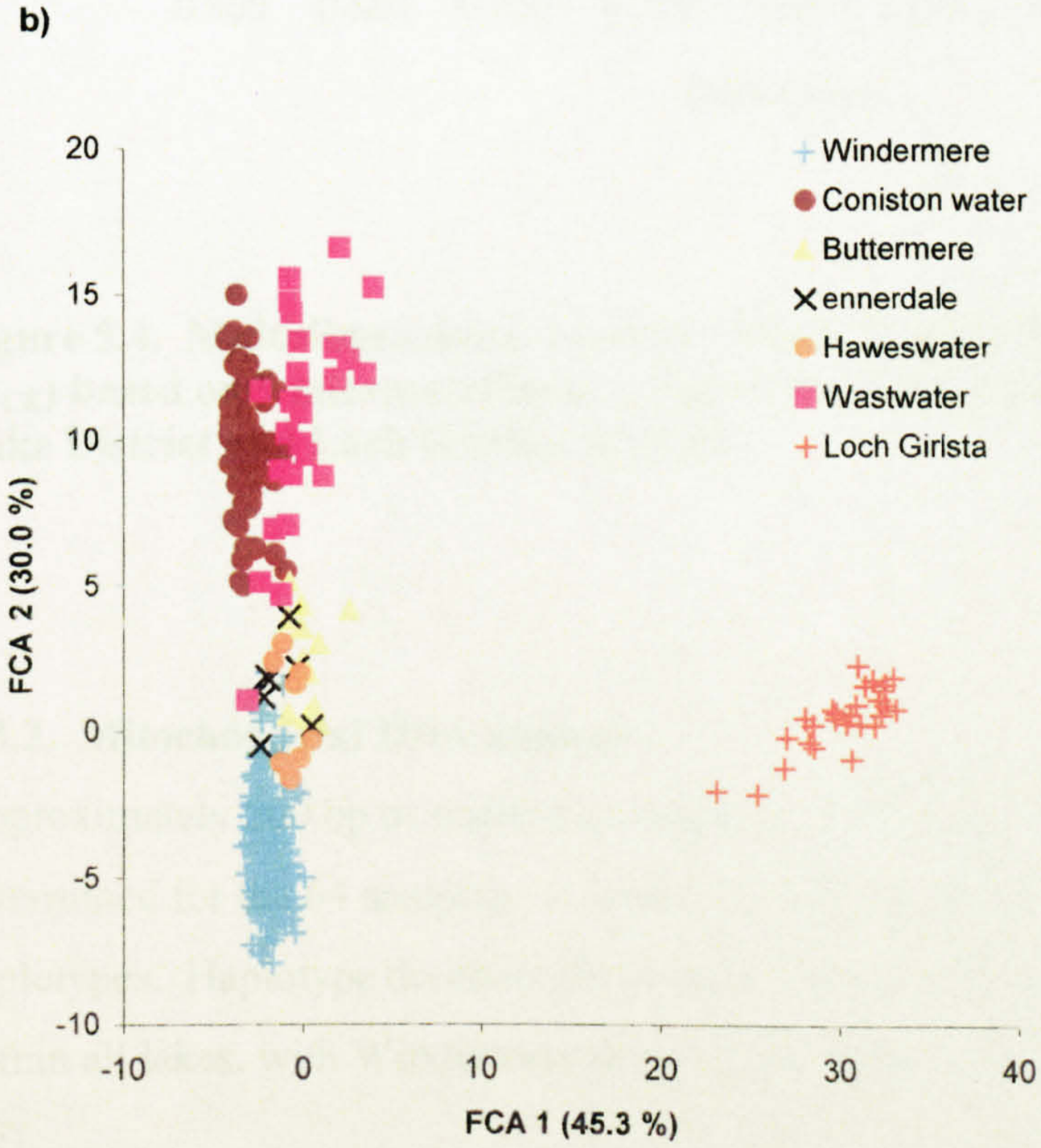


Figure 5.3. Factorial correspondence analysis (FCA) of microsatellite data from Arctic charr from lakes within the Lake District, UK and Loch Girlsta, Scotland, using a) all 10 loci and b) 9 loci, omitting Sfo334.

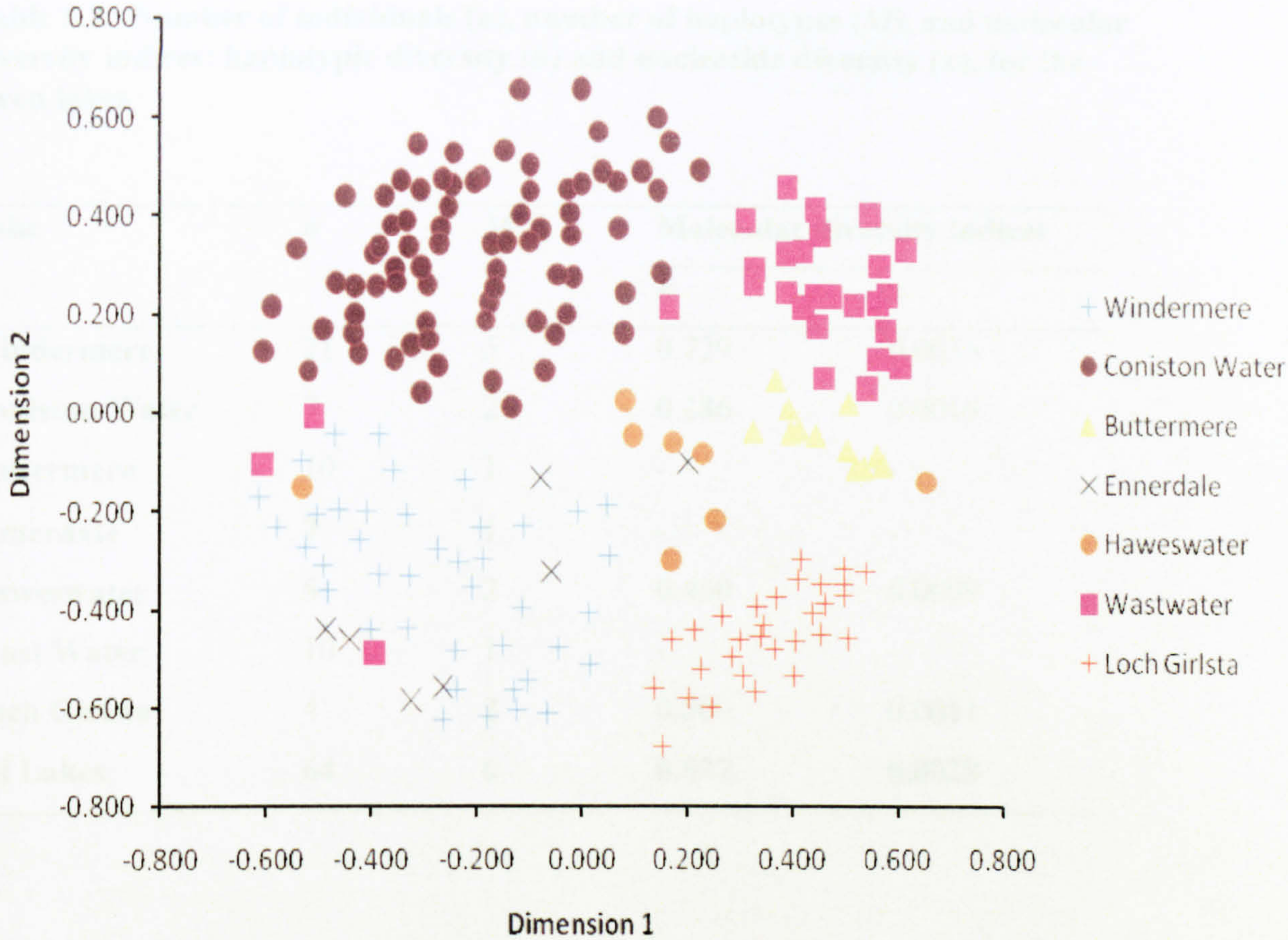


Figure 5.4. Multidimensional analysis (MDS) of individual genetic distances (D_{CE}) based on 9 microsatellite loci data from Arctic charr from lakes within the Lake District and Loch Girlsta, Scotland.

5.3.2. Mitochondrial DNA analysis

Approximately 500 bp of nucleotide sequence of the mtDNA control region was determined for the 64 samples. A total of five segregating sites (K), defined 6 haplotypes. Haplotype diversity (h) and nucleotide diversity (π) were both low within all lakes, with Windermere showing the highest values for both indices (Table 5.7).

Table 5.7. Number of individuals (*n*), number of haplotypes (*M*), and molecular diversity indices: haplotypic diversity (*h*) and nucleotide diversity (π), for the seven lakes

Lake	*n*	*M*	Molecular diversity indices	
			h	π
Windermere	21	5	0.729	0.0035
Coniston Water	7	2	0.286	0.0019
Buttermere	10	1	-	-
Ennerdale	7	1	-	-
Haweswater	5	2	0.400	0.0009
Wast Water	10	1	-	-
Loch Girlsta	4	2	0.500	0.0011
All Lakes	**64**	**6**	**0.577**	**0.0028**

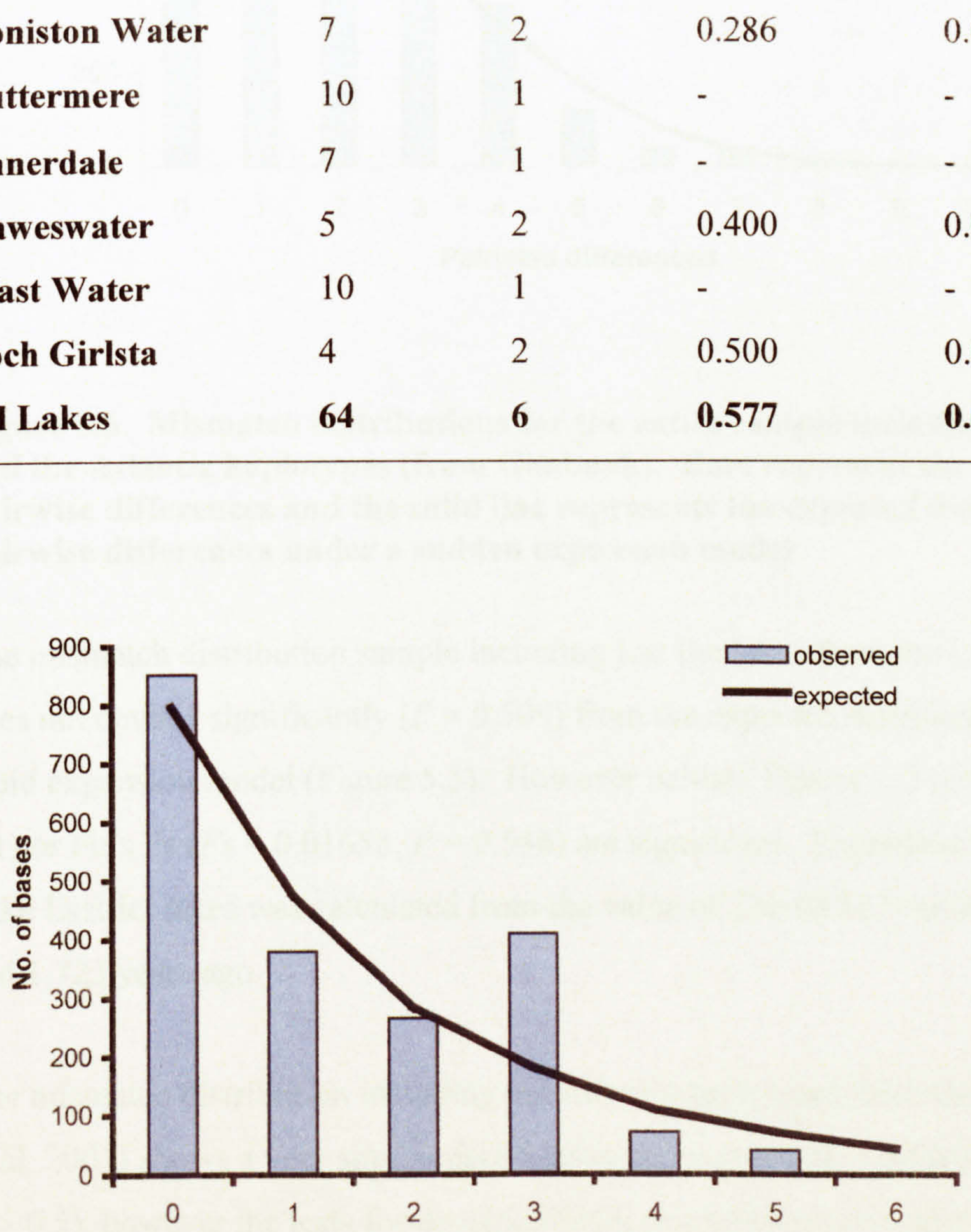


Figure 5.5. Mismatch distributions for the entire sample including all the lakes. Bars represent the observed pairwise differences and the solid line represents the expected distribution of pairwise differences under a sudden expansion model.

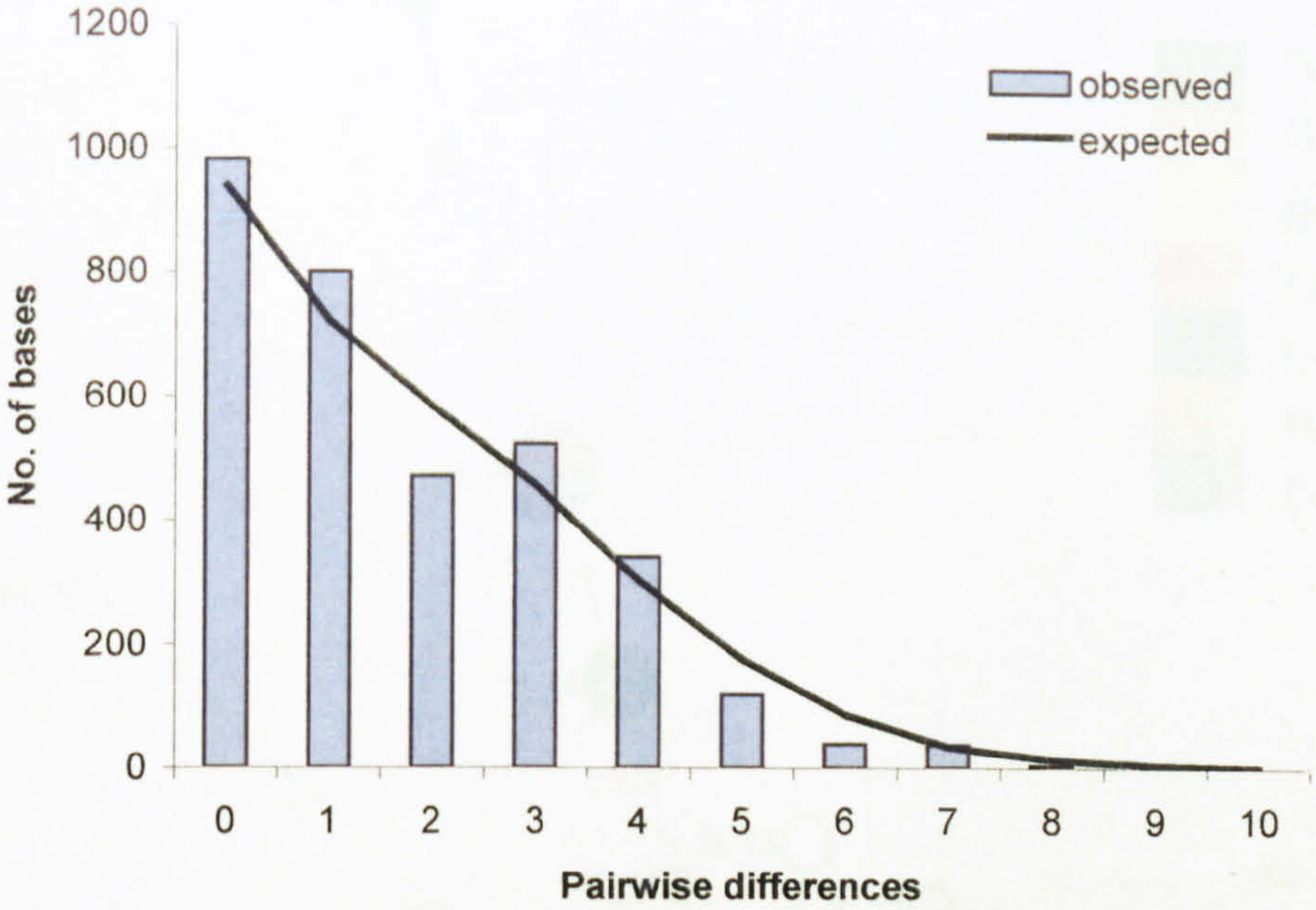


Figure 5.6. Mismatch distributions for the entire sample including all the lakes and the Atlantic haplotypes (from Genbank). Bars represent the observed pairwise differences and the solid line represents the expected distribution of pairwise differences under a sudden expansion model.

The mismatch distribution sample including just the lakes from the Lake District does not deviate significantly ($P = 0.509$) from the expected distribution under the rapid expansion model (Figure 5.5). However neither Tajima's D ($D = 0.526$; $P > 0.1$) or Fu's Fs ($Fs = 0.01658$; $P = 0.546$) are significant. Expansion time for the Lake District lakes was calculated from the value of *Tau* (0.517) as between 11,489 and 1,723 years ago.

The mismatch distribution including the Atlantic haplotypes from Genbank (Brunner et al. 2001) shows a very similar distribution and remains non-significant (Figure 5.6, $P > 0.5$), however the tests for deviation from neutrality are now highly significant ($D = -1.8121$, $P < 0.01$; $Fs = -8.7009$, $P < 0.001$). An estimated expansion time of between 72,888 and 10,933 years ago was calculated using *Tau* (3.28).

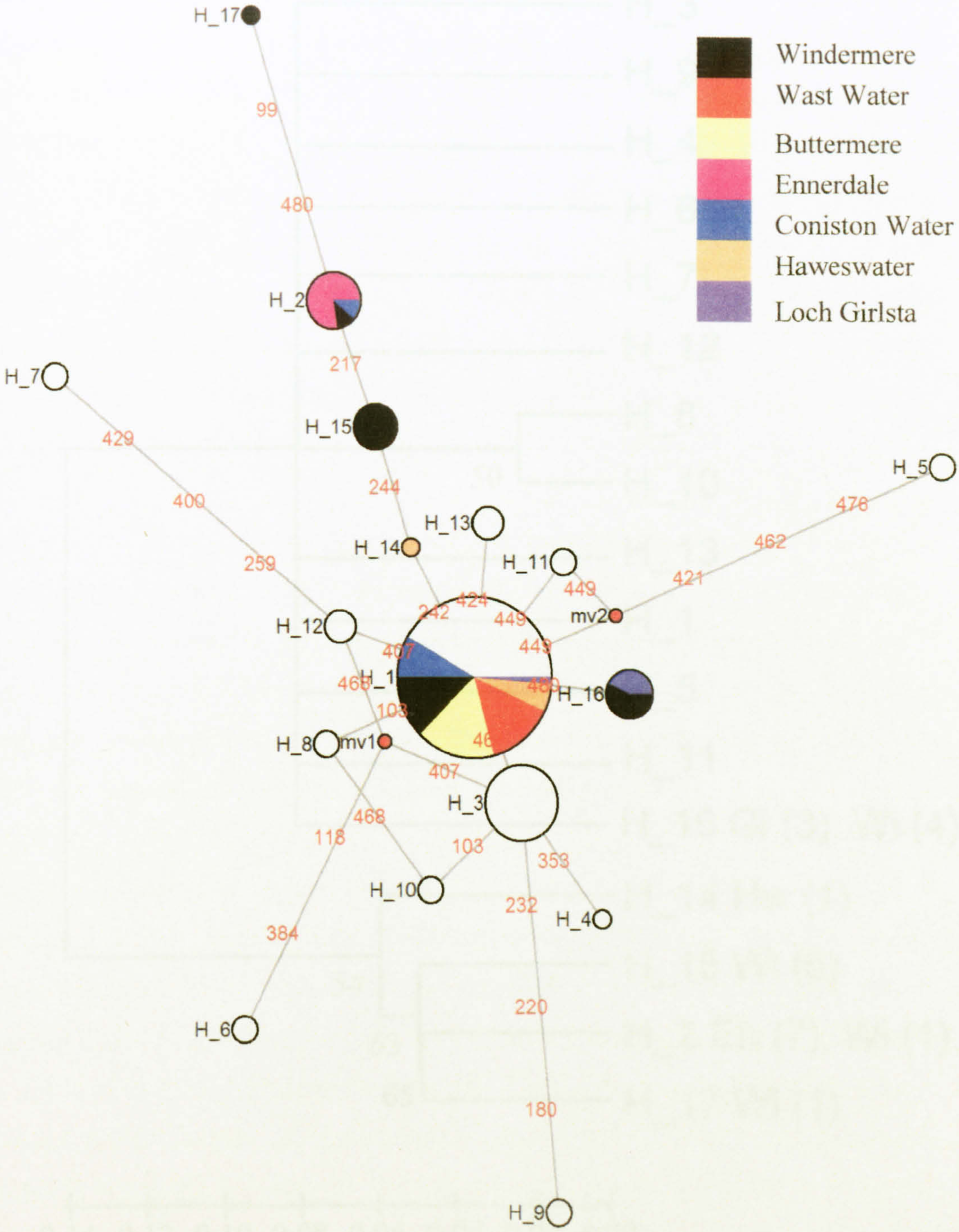


Figure 5.8. Median Joining spanning network of Arctic charr mtDNA haplotypes from the Lake District, UK, shown in colour (this study) and the Atlantic lineage haplotypes shown in white (Brunner et al. 2001).

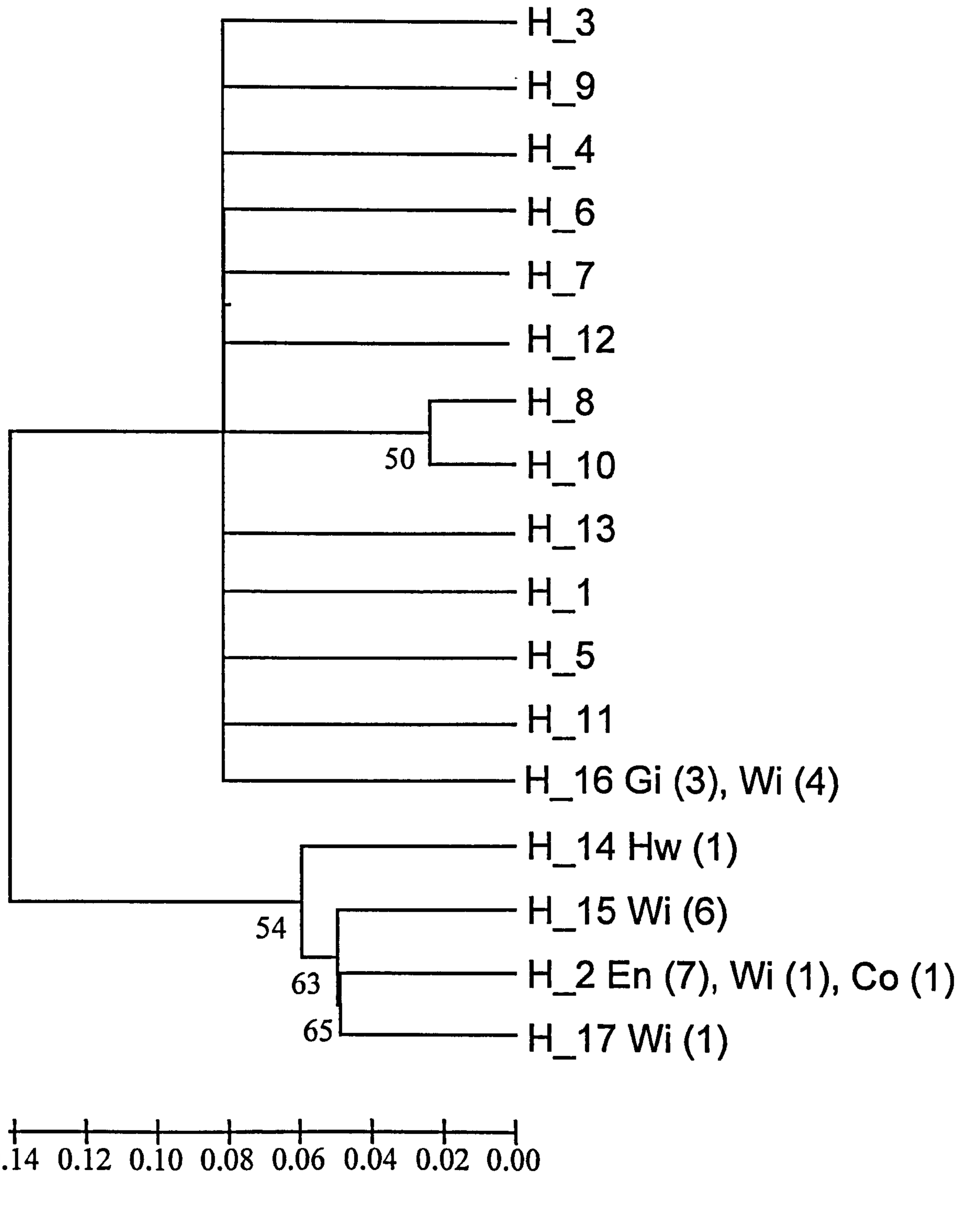


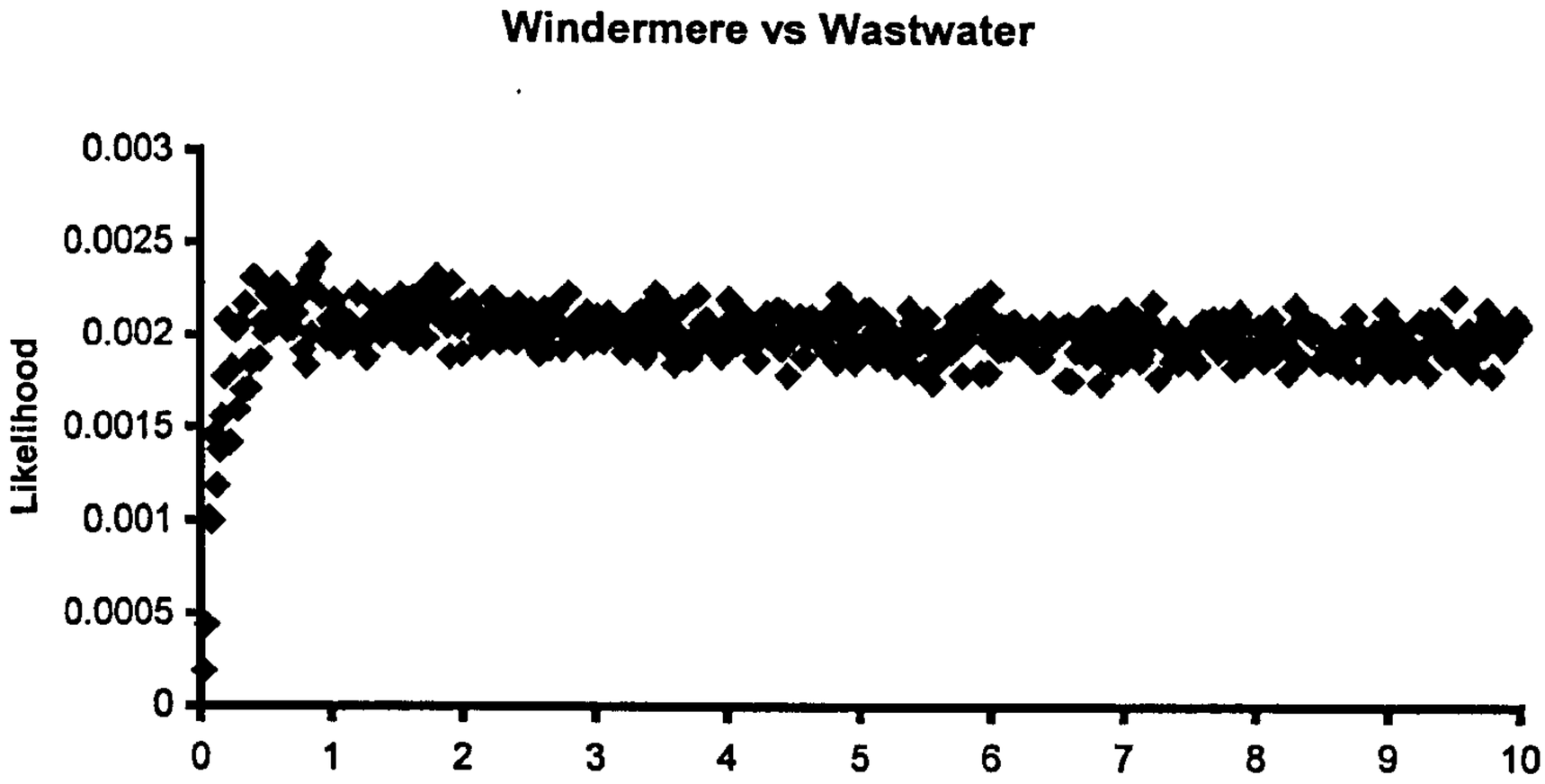
Figure 5.7. Linearised Neighbour-Joining phylogram constructed for Arctic charr mtDNA haplotypes from the Lake District UK and Arctic charr Atlantic mtDNA haplotypes taken from Brunner et al. (2001) (see Table 5.6 for haplotype locations). Co, Coniston Water; Wa, Wast Water; Wi, Windermere; Hw, Haweswater; Bu, Buttermere; En, Ennerdale; Gi, Loch Girlsta. Number of individuals of each haplotype are given in paranthesis.

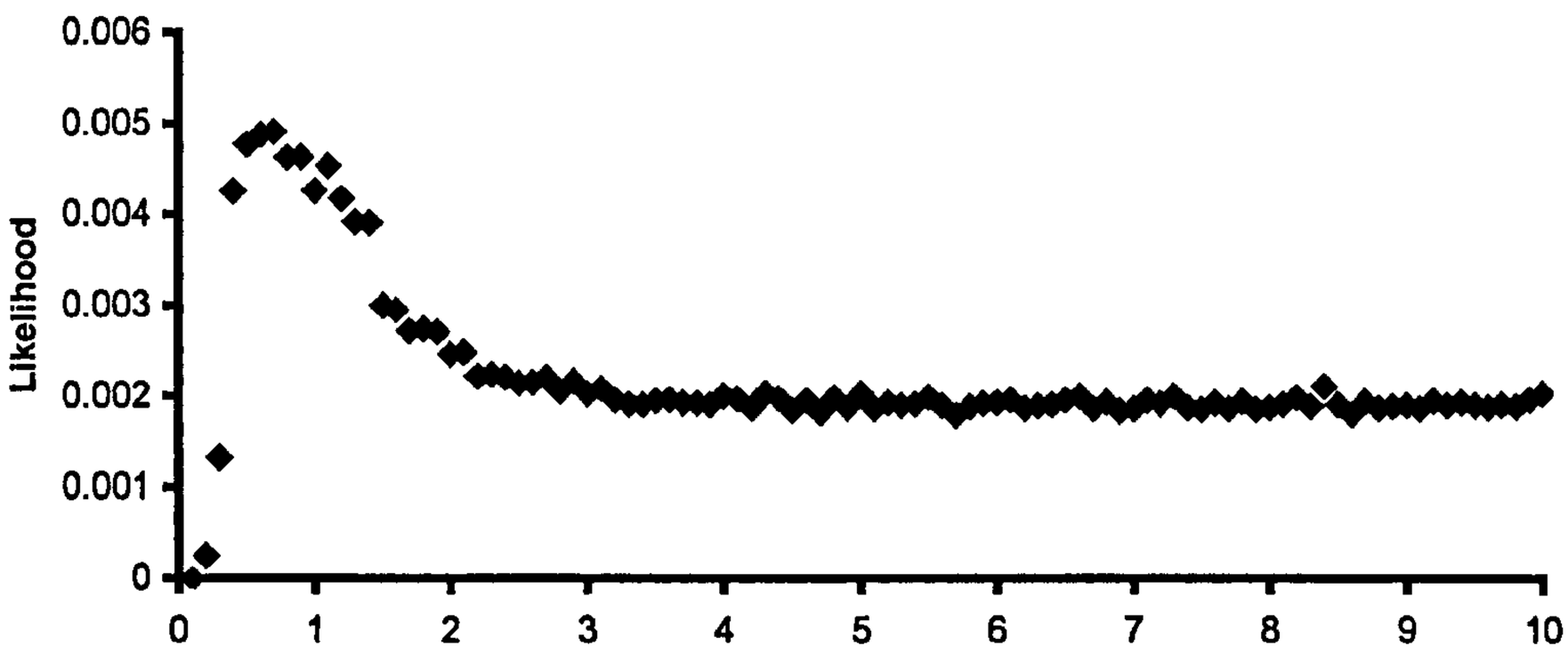
Of the six haplotypes found from the present study, one, H_1, was also present in the study by Brunner et al. (2001). This was by far the most abundant haplotype and was found in Loch Girlsta, in all of the lakes in the Lake District except for Ennerdale, as well as the Alpine region, Norway and Greenland (Table 5.3). No further haplotypes found in the Lake District were shared with those from either the 'Atlantic' or 'Arctic' lineage described by Brunner et al. (2001) (Tables 5.3 and 5.4). The neighbour-joining tree of mtDNA haplotypes shows very low distances between haplotypes with weak support for branches (Figure 5.7). The tree topography does however show a split between the haplotypes from Northern Europe and the North Atlantic, and the four haplotypes unique to the Lake District.

The median joining spanning network has a star shaped pattern consistent with expectations for a rapidly expanding population (Figure 5.8). The most common haplotype, H_1, found in the majority of lakes in the Lake District and in Alpine Europe, Norway and Greenland, can be seen in the centre, as the ancestral haplotype, with other haplotypes radiating from it. This reveals an expansion signal shared by all North Atlantic and Northern European populations studied to date.

Isolation with migration model parameters, θ, M, T and *TMRCA*, were estimated comparing Windermere and Coniston Water, Windermere and Wast Water, Coniston Water and Wast Water populations. The combined Lake District populations were also compared with haplotypes from the 'Arctic' and 'Atlantic' lineages (Brunner et al. 2001). The likelihood estimates of T for Windermere vs Wastwater, the Lake District vs the 'Atlantic' sequences and the Lake District vs 'Arctic' sequences are shown in Figure 5.10. Using the range of estimated mutation rates as 5×10^{-9} and 1×10^{-7}, t was calculated to be approximately 250,000 and 12,500 years ago for the divergence of 'Arctic' and Lake District sequences and 50,000 and 2,500 years ago for the divergence between the 'Atlantic' and Lake District sequences. Mdiv could not resolve a value of T for comparisons within the Lake District, possibly due to the low haplotype variability amongst these lakes (Figure 5.9). The time since most recent common ancestor was similar for all comparisons (*TMRCA* = 3.23-5.0) and corresponded to an age of between 200,000 and 7,500 years old. Migration rates

were higher between Coniston Water and Windermere ($M = 2.1$) and Coniston Water and Wastwater ($M = 1.2$) than between Windermere and Wastwater ($M = 0.3$).





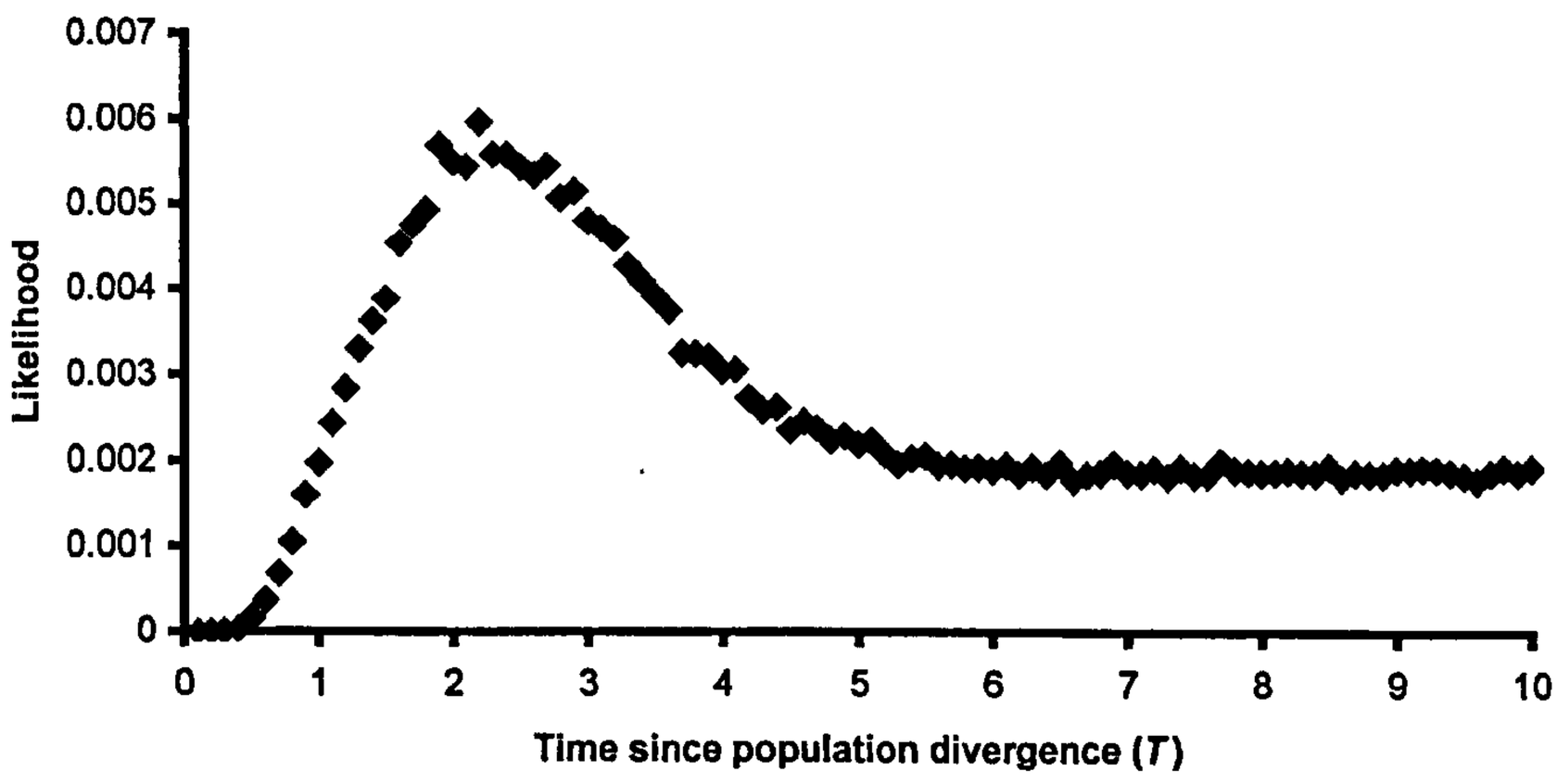


Figure 5.9. The likelihood estimates for the time since population divergence parameter (*T*) for analysis with Windermere vs Wastwater; Lake District vs Atlantic and Lake District vs Arctic.

5.4. Discussion

5.4.1. Genetic Diversity

The level of overall diversity indicated by microsatellite analysis in the Lake District (F_{ST} = 0.157) was lower than values for other regions, for example, Iceland and Scandinavia (F_{ST} = 0.263 and 0.360, respectively, Primmer et al. (1991) and the British Isles excluding England (F_{ST} = 0.260, Wilson et al 2004). On the other hand, this study was based on relatively fewer lakes, suggesting that a more expansive study of the Lake District may reveal genetic diversity comparable to other regions. High genetic differentiation between lakes due to genetic drift after founder events and subsequent low levels of gene flow is consistent with other microsatellite-based studies of land-locked populations of Arctic charr (Primmer et al. 1999; Bernatchez et al. 2002; Wilson et al. 2004).

The mitochondrial DNA analysis, on the other hand, has shown little microgeographic variation of Arctic charr within the Lake District where only six haplotypes were found amongst the six lakes sampled. The molecular diversity indices indicate low diversity (h = 0.577; π = 0.0028) compared with other European regions (Table 5.7). These values however are not as low as those of comparable landlocked lakes, such as those in Alpine Europe (Table 5.7). Such low levels of diversity are consistent with the effects of a population bottleneck during founder events, which would reduce source diversity (Avise et al. 1984). Brunner et al. (2001) also found that the nucleotide diversity for the whole of the 'Atlantic' lineage was very low compared to the 'Arctic' lineage, which had the highest value of all five lineages (Table 5.7). This suggests a loss of mtDNA diversity, likely during the repeated colonisations of new areas during repeated glacial retreats. Reduced genetic diversity compared to fish species from non-glaciated regions is a feature shared by many northern fish species (Bernatchez and Wilson 1998). For example, Bernatchez and Dodson (1994) compared levels of mtDNA diversity in Nearctic whitefish (*C. clupeaformis*) populations from North America with Palearctic whitefish (*C. lavaretus*) populations from Europe. The Palearctic whitefish populations had much higher levels of diversity due to the lesser impact of the Eurasian ice sheet, compared to those in North America.

Table 5.8. Haplotype diversity (h) and nucleotide diversity (π) for other European regions taken from Brunner et al. (2001).

Region	N	h	π
Lake District	64	0.577	0.0028
Alpine Europe	26	0.283	0.0005
Iceland	6	0.733	0.0017
Norway	16	0.835	0.0069
Atlantic Lineage	68	0.808	0.0047
Arctic lineage	38	0.952	0.0093

5.4.2. Genetic differentiation and geographic structure

In general, this study reveals significant genetic differentiation between the lakes within the Lake District studied, despite the short geographic distances between them (maximum distance < 50 km) (Table 5.6). This confirms that the lakes contain genetically distinct populations between which gene flow has been restricted and on which other evolutionary forces may be acting independently to enhance their divergence. The restriction of gene flow is not surprising given their locations within separate basins (Figure 5.1) and genetic differentiation in Arctic charr populations over short geographic scales has also been found in Iceland and Scotland where gene flow is limited by geographical barriers (Brunner et al. 1998, Bernatchez et al. 2002, Wilson et al. 2004). Unsurprisingly, Loch Girlsta showed the greatest degree of differentiation from all of the other lakes; due to its more northern location on Shetland Mainland, Scotland it is likely that Loch Girlsta was formed well after the Lake Distict lakes in a separate colonisation event (Wilson et al 2004).

Patterns of genetic structure in fishes are often related to the length of reproductive isolation between populations, consistent with historical and contemporary geographical structure, for example, isolation by distance (Castric and Bernatchez 2003, Primmer et al. 2006). In the case of postglacial fish populations, historical patterns of geneflow may also be inferred by patterns of deglaciation (Hewitt 1996).

Therefore, in the case of the Lake District, it may be expected that the genetic structure among the lakes may reflect patterns of glacial retreat and historical patterns of geneflow amongst lakes.

In general, during deglaciation events, the ice sheets move from south to north, however, the radial pattern of deglaciation that occurred in the Lake District meant that ice sheets moved back up valleys to the high point from which they were formed in the centre of the Lake District, at approximately the same time (Evans et al. 2005). At the same time it is possible there was some north/south divide, as the more northern lakes would have been influenced by ice from the northern ice sheet (D. Evans, pers. com.) (Figure 5.1). This could mean a somewhat earlier colonisation for the more southerly lakes (Windermere, Coniston Water and Wastwater), which could result in genetic differentiation if there was temporal differentiation within the source population. However neither the FCA (Figure 5.3) or the MDS analyses (Figure 5.4) suggested any differentiation between northern and southern lakes. If anything, there is a tendency for the smaller, northern lakes to group with Windermere. There is also no evidence within these results to suggest that Coniston Water underwent a bottleneck more recently than when the lakes were colonised, as was suggested by Child (1984).

A lack of structure based on geography, as is shown by the FCA analysis, is consistent with other studies of landlocked Arctic charr populations. Bernatchez et al. (2002) studied genetic differentiation between 12 landlocked populations of charr in Maine using six microsatellite loci, and also found significant divergence (maximum $F_{ST} = 0.17$) but no pattern based on geographic location. They concluded that these populations were colonised by the same ancestral population as the lakes became available following the retreat of the Wisconsin ice sheet. A similar pattern is likely to be true of the populations in the Lake District, which will have colonised the rivers and lakes, formed during the deglaciation.

Historical patterns of gene flow can also be inferred from geographical location. It is possible that these populations were anadromous during the time after their

colonisation. The climate during this period was likely to be similar to that in more northern latitudes at present, where Arctic charr populations remain anadromous (Klemetsen et al. 2003). It therefore seems likely that the colonising charr would utilise the newly formed lakes and rivers as spawning grounds whilst their smolts still returned to the sea to feed. Arctic charr lakes in northern areas of the species' range often, where access to the sea allows, have sympatric populations of anadromous and resident non-anadromous charr (Klemetsen et al. 2003). The factors determining the 'decision' to become anadromous is thought to be influenced by both plastic (relating to environmental cues such as food availability) and deterministic traits (McDowall 2001). Arctic charr populations in the Lake District are all non-anadromous resident populations despite there being substantive physical barriers to the sea in several lakes including Wastwater, Windermere and Coniston Water. It is therefore possible that these Lake District populations remained anadromous until Atlantic waters warmed.

Genetic studies of geographically proximate anadromous populations of Arctic charr at northern latitudes have found them to be less divergent than landlocked populations. Genetic differentiation among anadromous Arctic charr populations in Labrador, Canada, was found to be significant ($F_{ST} = 0.059$) based on six microsatellite loci, but low compared to the Lake District values (Bernatchez et al. 1998). These results were consistent with those reported in other anadromous fish species (DeWoody and Avise 2000) and have been attributed to their propensity to stray from their natal rivers when spawning. If an anadromous history is true for the Lake District population then a closer genetic relationship between those populations that are geographically close may be expected. For example, the rivers draining Windermere and Coniston Water share a tributary before flowing into Morecambe Bay, providing more opportunity for geneflow of anadromous fish between these catchments. The F_{ST} values (Table 5.6) and the neighbour joining tree (Figure 5.2) suggest some support for this, where the lower F_{ST} and a lineage cluster link Windermere and Coniston Water. However, the FCA and MDS analyses suggest similar differentiation between Windermere and either Coniston Water or Wastwater, and Wastwater drains to the sea further north (Figure 5.1). The pattern is therefore

not clearly resolved, though on balance may suggest some history of migration among populations sharing drainages to the south.

5.4.3. Phylogeography and divergence times

The fusion of population genetics and biogeography has made it possible to assess the impact of historical events on the genetic composition and structure of modern populations (Avise et al. 1987). Assessment of Arctic charr phylogeography within the Lake District and the relationship with other lineages has identified the possible historical events and migrations that have likely shaped its modern-day genetic structure.

The star-shaped median-joining spanning network (Figure 5.8) shows that the Lake District populations expanded from a haplotype common to Lakes from Norway, Greenland and Alps, and all of the Lake District lakes except for Ennerdale, suggesting that the 'Atlantic' lineage described by Brunner et al. (2001), which contained samples from Northern Europe and Canada (Table 5.3), expanded, along with the Lake District from a common ancestor. This is largely consistent with the microsatellite phylogeography described by Wilson et al. (2006) for lakes within Iceland, Scandinavia, Scotland and Ireland, where weak large-scale structuring confirmed that all regions were likely colonised by a single lineage, as had already been suggested by Brunner et al. (2001). The mtDNA neighbour-joining phylogram from this study also shows a similar pattern although, four out of the six haplotypes from the Lake District form a separate lineage from the 'Atlantic' lineage (Figure 5.7) with significant bootstrap support. Of the remaining two, one is the ancestral haplotype, H_1, and the other, H_16 is only found in Windermere and Loch Girlsta.

The Lake District therefore contains unique haplotypes evolved from an ancestral haplotype from the 'Atlantic' lineage described by Brunner et al. (2001). Brunner et al. (2001) also observed a positive correlation between genetic divergence and isolation by distance within the 'Atlantic' lineage and suggested that this supported a scenario of dispersal across the Atlantic via Greenland and Iceland to Continental Europe, from the edge of a rapidly expanding population, most likely from refugia in

the Arctic. The results from the present study, especially the common occurrence of H_1 in the Lake District, along with those from Brunner et al. (2001) and Wilson et al. (2006) suggest that the Lake District Arctic charr populations underwent a relatively recent population expansion from an ancestor common to the rest of Northern Europe. The mismatch distribution analysis of both the Lake District and 'Atlantic' lineage sequences also shows a signal for a recent population expansion (Figure 5.6). The divergence times between these two lineages, calculated from *Tau* (mismatch distribution analysis) and from *T* (Mdiv), were 70,000 to 3,500 and 50,000 to 2,500 years ago respectively. The temporal estimate for this expansion is broadly consistent with the time when habitat was released by retreating ice in the Lake District region.

Estimating divergence times from molecular data is a highly controversial issue due to considerable debate on the constancy of divergence rate estimates and the degree of error associated with their prediction (Hillis et al. 1996). The 'molecular clock' hypothesis states that the rate of molecular change is constant enough to determine times of divergence, however divergence rates are not homogeneous and can vary considerably among genes, among taxa, and among different sites in DNA sequences (Hillis et al. 1996). Phylogenetic divergence rate estimates for mtDNA are approximately 2 % per MY (e.g. Brown et al. 1979) but recent studies have estimated much higher rates of mtDNA control region evolution over Holocene timeframes (e.g. 100 % divergence per million years, Ho et al. 2007).

The divergence rates used were 5 % and 100 % sequence divergence per million years. These were chosen as they cover the full range of rates that have been found for the mtDNA control region. Rates at the lower end of this scale have been described for the control region in other fish species for example, sturgeon (*Acipenseridae*) (approximately 8 %, Brown et al. 1993). However lower control region mutation rates have also been observed in brook charr (*Salvelinus fontinalis*), a species closely related to Arctic charr. Bernatchez and Dodson (1993) found a transition/transversion ratio of 1.9:1 in brook charr relative to the ratio of 27:1 reported for sturgeon (Brown et al. 1993) but consistent with the ratio of 2:1 found in

brown trout (*Salmo trutta*) (Bernatchez et al. 1992). Bernatchez and Dodson (1993) concluded that transitional mutations may be more restricted in the control region of salmonids leading to a decrease in the mutation rate. Divergence rates are also thought to be variable through time (Ho et al. 2005). Ho et al. (2005) estimated the relationship between divergence rates and calibration points through time and found that avian control region rates were fairly constant at approximately 2% divergence per MY up until 1 MY ago when they rapidly increased up to the present.

Despite the wide ranges of divergence rates, the results give two clear expansion signals. The more ancient signal was dated between 250,000 and 12,500 years ago. As this is a more ancient expansion, the lower divergence rates are likely to be more accurate in this case (Ho et al. 2005) and this generally corresponds to the last interglacial period. The more recent signal is prior to the last glacial maximum, which corresponds to the formations of the lakes in the Lake District. Radiocarbon dating on sediment has dated Windermere's formation at <14,623 ± 360 radiocarbon years BP (Evans et al. 2005). This is consistent with the theory that the lakes were colonised by a common ancestral population from an Atlantic coastal refuge as has been described for Arctic charr from the Arcadia region in North America which were thought to have colonised northwards from refugia off the coast of Maine (Bernatchez and Dodson 1991). Geneflow between the lakes was likely ongoing as a result of anadromous migrations until this ceased as a result of temperature rises (Wilson et al 2004).

5.4.4. Implications for conservation and management

The results presented here have several implications for the management of Arctic charr in the Lake District. Firstly both the microsatellite and mtDNA results consistently indicate that the Lake District represents a monophyly that has evolved independently from populations in the rest of Europe. All the charr populations studied here therefore represent an evolutionary significant unit (Moritz 1994). Secondly, the division of Lake District charr into two species, based on spawning behaviour by Kottelat and Freyhoff (2007) needs revision. The results from this chapter show that each lake studied represents distinct gene pools and this, coupled

with the phenotypic variability described in Chapter Three, suggests that each population should be managed as a separate unit. Future management plans, therefore, should continue to recognise this and protect the populations separately. The alternative, to only manage the two putative species, suggested by Kottelat and Freyhof (2007), would result in a loss of genetic diversity.

Chapter 6 : General Discussion

The theory of ecological speciation in association with the adaptive evolution of species is a highly contentious issue in evolutionary biology. Adaptive radiations can be rapid and extensive, the most dramatic and well documented of which are the Galapagos finches and the East African cichlid fishes (Schluter 2001). Although the theory of ecological speciation is well documented, there are many gaps in the evidence available. Broadly this thesis aimed to use the study of Arctic charr populations to provide further understanding of firstly, the ecological processes important in the production of trophic polymorphisms, and secondly, the evolutionary mechanisms that cause their reproductive isolation and genetic differentiation in sympatry. More specifically, this thesis assessed the phenotypic and genetic variability both within and between several lakes in order to understand the origin and diversity of Arctic charr in the English Lake District and the implications for their effective conservation.

6.1. Origin and diversity of Arctic charr in the Lake District

Consistent with previous studies of this species, the results presented in Chapter Five indicate that the Arctic charr populations in the Lake District originate from a common ancestral population. Given the anadromous behaviour of charr in more northern latitudes, this ancestral population was also likely to have been anadromous, colonising the lakes from the Atlantic after they were formed. This is consistent with the recent expansion signal shown by the mitochondrial DNA analysis. A more ancient signal was also indicated for the divergence of the Lake District and Arctic sequences (Brunner et al. 2001), suggesting that a population expansion of Arctic charr also occurred in the last interglacial period.

The lakes are now geographically isolated from one another and, as expected, charr from the three main lakes studied, Windermere, Coniston Water and Wastwater, show significant genetic differentiation based on microsatellite DNA analysis. Given the degree of differentiation and the short time scale over which this has occurred,

the lakes were likely colonised by relatively small populations of charr, which were subject to strong genetic drift.

6.2. Ecological divergence in sympatry

6.2.1. Trophic Polymorphism

The development of trophic polymorphisms (morphotypes adapted to alternative niches within a population) occurs by divergent natural selection between environments, often mediated by competition for resources in two ways. Either intraspecific competition encourages the exploitation of different environments where contrasting selection pressures prevail, or the absence of competitive species allows diversification by ecological opportunity (Skulason et al. 1999). The ecological and evolutionary processes thought to give rise to trophic polymorphism related to resource availability are both intra- and interspecific competition (Skulason et al. 1999). The processes of character release and character displacement have been extensively reviewed in freshwater fish (Schluter 1996, Robinson et al. 2000). Studies on vertebrate taxa, including freshwater fishes, have shown that trophic polymorphism can originate and be maintained by density dependent or disruptive selection (Hori 1993, Smith 1993, Bolnick 2004, Jastrebski and Robinson 2004), however few studies have concentrated on Arctic charr.

Extensive literature on Arctic charr and other freshwater fish species (for example, whitefish, sunfish, and East African cichlids) has provided clear evidence for a relationship between diet and morphology (Malmquist et al. 1992, Hegrenes 2001, Andersson 2003, Parsons and Robinson 2007). Several factors suggest that there is an adaptive component to the development of polymorphisms. Firstly, variation in morphology is often functional and directly related to foraging success. This interpretation has been supported by experimental studies of foraging success (Andersson 2003, Parsons and Robinson 2007). Andersson (2003), for example, showed that benthic morphs of Arctic charr were less successful feeding on pelagic prey and vice versa for the pelagic morphs. Secondly, the patterns of phenotypic variability of Arctic charr within single lakes is replicated in many but not all such

lakes in which they are found, and is strongly linked to the utilisation of divergent niches, namely pelagic and benthic habitats.

The Arctic charr populations in the English Lakes also show a high degree of phenotypic variability amongst lakes (Figure 3.2), however, Coniston Water was the only lake studied to show a large degree of within-lake variation. On the other hand, low sample sizes from Ennerdale, Buttermere and Haweswater prevented meaningful examination of their phenotypic variability and increased sampling effort may well find polymorphisms within these lakes as well.

The variation of morphology of charr within Coniston Water was consistent with that observed in charr systems throughout their range, and coincided with the utilisation of the pelagic and benthic niches (Schluter 1996). Dietary analysis on the other hand indicated little segregation between the morphs although it did indicate that the pelagic morph was specialised, feeding on zooplankton only, whereas the benthic morph was more generalist, feeding on both pelagic and benthic prey. It is also highly possible that this limited degree of dietary segregation is more a factor of sampling, as although all the fish were caught in benthic nets, they were caught in June when zooplankton was likely to be abundant. Other studies have documented seasonal niche shifts by morphs in systems at higher latitudes (Klemetsen and Grotnes 1975, Andersson et al. 2005), which is another possibility to explain the pattern observed in Coniston Water. Year-round dietary analysis of both morphs, along with stable isotope analysis of muscle tissue would provide more in-depth information on the dietary segregation of these morphs.

The morphology of Windermere charr is broadly similar to that of the pelagic morph found in Coniston Water, and would be consistent with foraging behaviour in the pelagic niche. This phenotype was consistent with the diet of Windermere charr described by Frost (1977) that primarily consisted of zooplankton prey for much of the year, with a switch to benthic invertebrates over the winter and a strong importance for charr eggs during the spawning period. Chapter Two however describes a change in dietary composition in the present study. Valid comparisons,

made for the months of March, June and November, indicated an increased importance of benthic invertebrates across seasons in the present diet. Although it is difficult to determine the cause of the change in diet, several factors have been discussed, including competition for resources by zooplanktivorous fish species and changes in prey population density. If phenotype is induced by foraging behaviour as has been indicated by some experimental studies in Arctic charr, then this dietary shift would be expected to result in a change in morphology. This has not been the case, suggesting either that morphology is stable over time regardless of changing diet, or that the association between morphology and diet is less deterministic in this lake system than elsewhere. A stable morphology over time would be evidence of the genetic assimilation of traits associated with feeding (Pfennig et al. 2007). In time, given strong selective pressure, possibly caused by the competitive interactions of zooplanktivores, this may theoretically cause either the competitive exclusion of Arctic charr or character displacement resulting in a shift in mean phenotype (Rice and Pfennig 2007). On the other hand, flexibility of diet is common in many populations (Hindar and Jonsson 1993) and morphological differences may only have clear function in cases of extreme specialisation (Snorrason et al. 1994).

Generally, the most complex charr systems are found in deep Arctic lakes that are relatively depauperate of other fish species, allowing the assumption that 'ecological opportunity' has allowed phenotypic divergence, to be made (Klemetsen et al. 1997, Gislason et al. 1999, Skulason et al. 1999). In the case of these lakes, Wastwater is the most oligotrophic (Maberly et al. 2003) but, although information on its fish community is limited, it is thought to support brown trout, minnow, and three-spined stickleback as well as Arctic charr (Winfield et al. 2006). Coniston Water on the other hand is mesotrophic but is thought to support only small populations of trout and pike, whereas littoral species, such as minnow and perch are more abundant (Winfield et al. 2004c). Windermere is the most eutrophic lake and supports a much more diverse fish community (Winfield et al. 2005). The comparison of these three lakes could also indicate a link between the development of resource polymorphism, lake productivity and fish community structure. Coniston Water, the only lake studied that supports two charr morphs, is thought to have smaller populations of

possible competitors for example, brown trout (Forseth et al. 2003), which is present in Wastwater. Coniston Water also does not have large populations of introduced species, especially roach as is the case in Windermere. In-depth knowledge of these fish communities is lacking however, and further investigation of species abundances within these lakes is required.

A more exhaustive survey of lakes throughout the range of Arctic charr would give a better understanding of the environmental factors important in the development of resource polymorphism in this species. Comparisons between lakes housing only one morph and those where several morphs occur would be especially useful. Environmental parameters, such as lake depth, area, altitude, latitude, habitat diversity, fish community complexity and productivity should be measured in order to ascertain any correlations between environmental and biological factors and the extent of divergence.

6.2.2. Reproductive isolation and genetic differentiation

In many cases phenotypic divergence is coupled with the evolution of reproductive isolation and the genetic divergence of alternative morphs, although the extent of genetic differentiation varies widely among systems (Skulason et al. 1999). In freshwater fish, genetic studies of sympatric and allopatric forms have suggested that sympatric morphs of the same species do have a sympatric origin and do not represent separate invasions of allopatric populations (Taylor and Bentzen 1993). The same is true for the Arctic charr in the Lake District, where mitochondrial DNA shows a common ancestor for all the lakes (Chapter Five). Therefore genetically differentiated sympatric morphs have in the cases studied here diverged within the lake since their colonisation.

Trophic polymorphisms and the use of different habitats alone are unlikely to result in reproductive isolation, unless the phenotypic traits are linked to variation in breeding behaviour. Reproductive isolation within a system can be caused by variation in spawning locations and philopatry, allochrony or strong assortative mating on breeding grounds. Weak but significant genetic divergence based on

spawning time and location, occurs in the Windermere charr population (Chapter Four). Trophic polymorphism is not pronounced in Windermere, although it is possible that spawning behaviour was linked to morphology in the past. In this case, trophic polymorphism may have been an intermediate step in the production of these populations, but since diminished, possibly due to changing environmental conditions while the different spawning habits were retained. Trophic polymorphisms have been suggested as an intermediate step in the ecological speciation of closely related sympatric cichlid species that do not currently exhibit resource polymorphism (Meyer 1993).

The Arctic charr populations in Coniston Water also exhibit weak genetic differentiation, less so than in Windermere, therefore reproductive isolation appears to be incomplete. Despite local opinion suggesting the presence of both autumn and spring-spawning charr in Coniston Water, intensive netting by Frost (1977) showed that spawning occurred in the spring only, although the exact intensity or netting methods are not described. Arctic charr require stony substrate that is free of vegetation and silt for spawning, which in most lakes limits the availability of spawning grounds. Although there is no known variation in the location of spawning grounds in Coniston Water, it is possible that different morphs spawn in different locations or different depths within the lake and weak homing tendency allows the mixing of the gene pools. Depth also appears to be a factor in Windermere, especially in the south basin, where the association between spawning season and depth is greater. Whitefish and grayling have been documented to exhibit large variation in depth of spawning grounds (Koskinen et al. 2001, Næsje et al. 2004, Ostbye et al. 2005). Further investigation into the spawning behaviour of Arctic charr within Windermere, especially the association with depth, would be useful for deeper understanding of the processes producing reproductive isolation.

Mate choice on spawning grounds is another possible mechanism resulting in reproductive isolation. Assortative mating of like-morphs has been observed in three-spined sticklebacks (Gudbjorg et al. 2006, Vines and Schluter 2006), several cichlid species (Knight and Turner 2004), as well as species of charr such as

Japanese charr (Maekawa et al. 1994), but has not been demonstrated in Arctic charr. In addition to prezygotic isolating mechanisms, postzygotic isolating mechanisms such as the reduced fitness of hybrids or reinforcement have been identified (Noor 1999). For instance, experimental studies on cichlid species have indicated reduced hybrid fitness in relation to colour and mate selection (Turner 2007). This process requires the phenotypic differences to have a genetic basis and that selective pressures are such that reduced fitness occurs. The absence of intermediate phenotypes within Coniston Water (Figure 3.8, Chapter Three) could be evidence of reinforcement but an inherent genetic basis would also have to be proven through breeding experiments and incomplete reproductive isolation between these morphs strongly suggests this is not the case. Although reduced foraging ability of morphs of Arctic charr on alternative diet has been suggested experimentally, this is not necessarily evidence of reduced fitness.

The patterns of genetic differentiation in the Lake District indicate less of an association with morphological divergence than has been described elsewhere (Gislason et al. 1999, Westgaard et al. 2004, Knudsen et al. 2006, Adams et al. 2007). Interestingly the morphs in Coniston Water exhibit only weak genetic differentiation despite strong morphological divergence, suggesting incomplete reproductive isolation. Although population differentiation can be an important step in the process of speciation, speciation is not an inevitable outcome of even extreme phenotypic differentiation. Strong phenotypic divergence without genetic divergence has been documented in other fish species, for example the Trinidadian guppy, where sneaky mating of males is sufficient to prevent genetic divergence (Magurran 1999).

In contrast, in Windermere, significant differentiation can be seen between spawning seasons and between the two lake basins that is not largely associated with morphology (Chapter Four). Complete reproductive isolation of populations in sympatry can be disadvantageous if this reduces the genetic diversity, which would be expected in a closed system as a consequence of reduced effective population sizes. This may make some level of ongoing gene flow adaptive. In this study

comparisons were based primarily on equilibrium models, which don't distinguish between low levels of gene flow and recent isolation.

It is already known that differing degrees of both genetic and phenotypic divergence occur throughout the range of Arctic charr, and that this is associated with a varying relationship between divergence and the environmental and genetic effects on development. The results from Windermere and Coniston Water add to the knowledge of such systems by providing information from lakes at the more southern boundary of its range. The weak divergence exhibited in both these lakes relative to others in more northern latitudes may suggest that contemporary selective pressures in these temperate lakes are likely to be different to those in Arctic or sub Arctic conditions.

6.3. Genetic variability in the Lake District and implications for conservation and management

The use of both mitochondrial and microsatellite DNA analysis was used to determine the genetic diversity and origin of Arctic charr populations within the Lake District (Chapter Five). The mitochondrial DNA analysis confirmed the hypothesis that Arctic charr from the Lake District lakes shared a common ancestor that colonised the lakes after they were formed during a rapid population expansion after the last glacial maximum. Although the dates given must be interpreted with caution, as discussed in Chapter Five, they correspond approximately to the age of Windermere from carbon dating (Evans et al. 2005). The monophyly of Lake District lakes (Figure 5.7, Chapter Five) is consistent with shallow mtDNA divergence among populations of Arctic charr from other regions, such as in Scotland (Wilson et al. 2004). This suggests that Arctic charr from the Lake District should be defined as an Evolutionary Significant Unit (EIU, Moritz 2004). The microsatellite DNA analysis however, shows that each lake studied was genetically differentiated. They therefore represent distinct gene pools and should be managed as such. The suggestion that the Lake District populations should be divided into two species based on spawning season and morphology as suggested by Kottelat and Freyhof (2007) is inconsistent with these results.

Any future management plans for Coniston Water should also take into consideration the newfound phenotypic and genetic variability described here in order to maintain diversity within this lake. Management procedures are already in place in Windermere, but future management plans should be sure to maintain their consideration of both autumn and spring-spawning populations. This present study has also highlighted some changes to the spawning grounds that were suggested by Frost (1965). Spawning grounds such as Rough Holme (J Fletcher, pers. com.) and Rawlinson's Nab (Partington and Mills 1988) are now used by both spring and autumn-spawners. One possible reason for this may be that suitable spawning grounds have declined, possibly due to increased vegetation growth or siltation. It is also possible that spawning had always occurred here, and possibly at other sites as well, but was not previously recorded. Frost (1965) described sites where spawning was assumed to take place but did not clearly describe where and with what effort these, and other potential spawning sites, were assessed. This lack of existing knowledge and the possible threats to spawning habitat highlight the need for a revised assessment of existing potential spawning habitat, including those grounds currently used.

6.4. Limitations and recommendations for future directions

The overall aim of this study was to provide further insight into the nature of phenotypic polymorphisms in Arctic charr and their reproductive isolation within and among the English lakes. This involved the examination of diet, morphology and genetics. One of the major limitations in the assessment of morphology and habitat use of Arctic charr in these lakes was that all of the individuals were caught using benthic nets and the habitat use of individuals could only be inferred from capture location and stomach contents. A useful extension of this aspect of the present study would be an assessment of habitat use of charr especially in Coniston Water where two distinct morphs were found. More extensive sampling of different habitats and the use of tracking methods such at telemetry could be useful in providing more accurate information on habitat use (Bourke et al. 1999, Babaluk et

al. 2001). Dietary analysis of Coniston Water and Windermere would also benefit from further temporal stable isotope analyses to assess the long-term diet of morphs.

Genetic studies in particular are affected by small sample sizes as this may affect allelic distribution and their frequencies and be misrepresentative of the true population. Future work should include more intensive sampling especially within Coniston Water where little genetic differentiation was detected. Larger sample sizes and the consequent increased power would facilitate the assessment of migration rates between morphs, though this becomes increasingly difficult to estimate as the migration rate increases. Also an increased knowledge of migrations between spawning locations in Windermere would be useful in interpreting the patterns of genetic differentiation. Philopatry, for example, may not be equal between the sexes. Telemetry could be used to help assess philopatry as has been used in studies of other taxa (McCubbing et al. 1998, Stewart et al. 2004).

It appears obvious that when phenotypic traits are differentially associated with seasonal or spatially isolated spawning sites this will result in the genetic divergence of different morphs, however, reproductive isolation can occur without isolation among spawning sites. The processes by which this may occur have been suggested based on knowledge of other species, but the exact processes important in reproductive isolation in Arctic charr remain unclear. It was beyond the scope of this study but future studies could concentrate on laboratory experiments to test the fitness of morphological hybrids, and this may increase understanding of the role of reinforcement. Similarly, laboratory mating trials would provide information on mate selection on breeding grounds.

The range of studies illustrating the large number of organisms exhibiting phenotypic plasticity highlights the advantages of this strategy, and poses the question as to why not all organisms display this trait (Price et al. 2003, West-Eberhard 2003, Nussey et al. 2007). Many reviews have examined the possible trade-offs and costs of plasticity (DeWitt et al. 1998). Experiments of selection in nature over time (Reznick and Ghalambor 2005), such as replicated introductions of individuals into

new environments, allows the study of patterns of plasticity, rates at which populations become differentiated, and the associated trade-offs (Ghalambor et al. 2007).

The stability of reproductive isolation between divergent morphs over time is poorly understood, and the potential for changing environmental conditions to affect the mechanisms involved is great. The breakdown of reproductive isolation between species pairs of three-spined sticklebacks has been attributed to increased water turbidity affecting female mate selection (Gow et al. 2006). Similar problems could be encountered in other species especially those in Arctic or sub Arctic lakes which may be greatly affected by factors associated with climate change (Reist et al. 2006). Windermere provides an opportunity to study the interactions between established and introduced species in a temperate system. The further assessment of the population dynamics of both Arctic charr and roach will increase understanding of the impacts of introduced species on temperate lake systems. Generally, ongoing studies that incorporate environmental parameters and population genetic studies, interpreted in the context of environmental change, will help provide information on the effects these factors could have on Arctic charr populations.

References:

Adams, C., D. Fraser, A. J. Wilson, G. Alexander, M. M. Ferguson, and S. Skulason. 2007. Patterns of phenotypic and genetic variability show hidden diversity in Scottish Arctic charr. Ecology of Freshwater Fish **16**:78-86.

Adams, C., D. J. Fraser, I. McCarthy, S. Shields, S. Waldron, and G. Alexander. 2003. Stable isotope analysis reveals ecological segregation in a bimodal size polymorphism in Arctic charr from Loch Tay, Scotland. Journal of Fish Biology **62**:474-481.

Adams, C., D. J. Hamilton, I. McCarthy, A. J. Wilson, A. Grant, G. Alexander, S. Waldron, S. S. Snorrason, M. M. Ferguson, and S. Skulason. 2006. Does breeding site fidelity drive phenotypic and genetic sub-structuring of a population of Arctic charr? Evolutionary Ecology **20**:11-26.

Adams, C. and F. A. Huntingford. 2002a. The functional significance of inherited differences in feeding morphology in a sympatric polymorphic population of Arctic charr. Evolutionary Ecology **16**:15-25.

Adams, C. and F. A. Huntingford. 2004. Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. Biological Journal of the Linnean Society **81**:611-618.

Adams, C. E., D. Fraser, F. A. Huntingford, R. B. Greer, C. M. Askew, and A. F. Walker. 1998. Trophic polymorphism amongst Arctic charr from Loch Rannoch, Scotland Journal of Fish Biology **52**:1259-1271.

Adams, C. E. and F. A. Huntingford. 2002b. Inherited differences in head allometry in polymorphic Arctic charr from Loch Rannoch, Scotland. Journal of Fish Biology **60**:515-520.

Ahas, R. 1999. Long-term phyto-, ornitho- and ichthyophenological time-series analyses in Estonia. International Journal of Biometeorology **42**:119-123.

Alabaster, J. S. and R. Lloyd. 1980. Water quality criteria for freshwater fish. Butterworths, London, UK.

Alexander, G. D. and C. E. Adams. 2004. Exposure to a common environment erodes inherited between-population trophic morphology differences in Arctic charr. Journal of Fish Biology **64**:253-257.

Amundsen, P.-A. 1995. Feeding strategy of Arctic charr (*Salvelinus alpinus*): General opportunist, but individual specialist. Nordic Journal of Freshwater Research **71**:150-156.

Amundsen, P.-A., H.-M. Gabler, and F. J. Staldvik. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data-modification of the Costello (1990) method. Journal of Fish Biology **48**:607-614.

Amundsen, P.-A., R. Knudsen, A. Klemetsen, and R. Kristoffersen. 2004. Resource competition and interactive segregation between sympatric whitefish morphs. Annales Zoologici Fennici **41**:301-307.

Anderson, M. J. 2003. DISTLM forward: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection. Department of Statistics, University of Auckland, New Zealand.

Andersson, J. 2003. Effects of diet-induced resource polymorphism on performance in arctic charr (*Salvelinus alpinus*). Evolutionary Ecology Research **5**:213-228.

Andersson, J., P. Bystrom, L. Persson, and A. M. De Roos. 2005. Plastic resource polymorphism: effects of resource availability on Arctic charr (*Salvelinus alpinus*) morphology. Biological Journal of the Linnean Society **85**:341-351.

Avise, J. C., J. Arnold, R. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. Annual Review of Ecology and Systematics **18**.489-522

Avise, J. C., J. E. Neigel, and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. Journal of Molecular Evolution **20**:99-105.

Babaluk, J. A., H. R. Wissink, B. G. Troke, D. A. Clark, and J. D. Johnson. 2001. Summer Movements of Radio-Tagged Arctic Charr (*Salvelinus alpinus*) in Lake Hazen, Nunavut, Canada. Arctic **54**:418-424.

Balon, E. K. 1984. Life histories of Arctic charrs: an epigenetic explanation of their invading ability and evolution. Pages 109-141 *in* L. Johnson and B. L. Burns, editors. Biology of the Arctic charr, Proceedings of the International Symposium on Arctic Charr. University of Manitoba Press, Winnipeg, Manitoba.

Baroudy, E. and J. M. Elliott. 1994. The critical thermal limits for juvenile Arctic charr, *Salvelinus alpinus*. Journal of Fish Biology **45**:1041-1053.

Beerli, P. and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population sizes in two populations using a coalescent approach. Genetics **152**:763-773.

Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. Journal of Fisheries Research Board Canada **29**:639-671.

Belkhir, K. 1999. Genetix, v. 4.0. Laboratoire Genome, Polpulations, Interactions, CNRS UPR, Montpellier, France.

Bell, M. A., W. E. Aguirre, and N. J. Buck. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution **58**:814-824.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and D. L. Wheeler. 2007. Genbank. Nucleic Acids Research **35**:D21-D25.

Bernatchez, L., J. B. Dempson, and S. Martin. 1998. Microsatellite gene diversity analysis in anadromous arctic char, Salvelinus alpinus, from Labrador, Canada. Canadian Journal of Fisheries and Aquatic Science **55**:1264-1272.

Bernatchez, L. and J. J. Dodson. 1990. Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial-DNA restriction analysis. Evolution **44**:1263-1271.

Bernatchez, L. and J. J. Dodson. 1991. Phylogeographic Structure in Mitochondrial DNA of the Lake Whitefish (*Coregonus clupeaformis*) and Its Relation to Pleistocene Glaciations. Evolution **45**:1016-1035.

Bernatchez, L. and J. J. Dodson. 1994. Phylogenetic relationships among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. Canadian Journal of Fisheries and Aquatic Science **51 (Suppl. 1)**:240-251.

Bernatchez, L., R. Guyomard, and F. Bonhomme. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout, *Salmo trutta* populations. Molecular Ecology **1**:161-173.

Bernatchez, L., J. G. Rhydderch, and F. W. Kircheis. 2002. Microsatellite gene diversity analysis in landlocked Arctic char from Maine. Transactions of the American Fisheries Society **131**:1106-1118.

Bernatchez, L. and C. C. Wilson. 1998. Comparative phylogeography of NeArctic and PaleArctic fishes. Molecular Ecology **7**:431-452.

Blackie, C. T., D. J. Weese, and D. L. J. Noakes. 2003. Evidence for resource polymorphism in the lake charr (*Salvelinus namaycush*) population of Great Bear Lake, Northwest territories, Canada. Ecoscience **10**:509-514.

Bolnick, D. I. 2004. Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. Evolution **58**:608-618.

Bone, Q., N. B. Marshall, and J. H. S. Blaxter. 1999. Biology of Fishes. Second edition. Stanley Thornes Ltd., Cheltenham.

Bourke, P., P. Magnan, and M. Rodriguez. 1997. Individual variation in habitat use and morphology in brook charr. Journal of Fish Biology **51**:783-794.

Bourke, P., P. Magnan, and M. Rodriguez. 1999. Phenotypic responses of lacustrine brook charr in relation to the intensity of interspecific competition. Evolutionary Ecology **13**:19-31.

Brown, J. R., A. T. Beckenbach, and M. J. Smith. 1993. Intraspecific DNA Sequence Variation of the Mitochondrial Control Region of White Sturgeon (*Acipenser transmontanus*). Molecular Biology and Evolution **10**:326-341.

Brown, W. M., J. George, M, and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences **76**.

Brunner, P. C., M. R. Douglas, and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr, *Salvelinus alpinus* (Teleostei: salmonidae) from central Alpine lakes. Molecular Ecology **7**:209-223.

Brunner, P. C., M. R. Douglas, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. Evolution **55**:573-586.

Bush, V. and C. E. Adams. 2007. Using phenotypic variation to determine conservation value: application of a novel approach to Arctic charr. Ecology of Freshwater Fish **16**:29-33.

Castric, V. and L. Bernatchez. 2003. The Rise and Fall of Isolation by Distance in the Anadromous Brook Charr (*Salvelinus fontinalis* Mitchill). Genetics **163**:983-996.

Castric, V., L. Bernatchez, K. Belkhir, and F. Bonhomme. 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus fontinalis* Mitchell (Pisces, Salmonidae): a test of alternative hypotheses. Heredity **89**:27-35.

Cavalli-Sforza, L. L. and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics **19**:233-257.

Child, A. R. 1984. Biochemical polymorphism in charr (*Salvelinus alpinus* L.) from three Cumbrian lakes. Heredity **53**:249-257.

Colosimo, P. F., C. L. Peichel, K. S. Nereng, B. K. Blackman, M. D. Shapiro, D. Schluter, and D. M. Kingsley. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. Public Library of Science **2**:0635-0641.

Coyne, J. A. and H. A. Orr. 1999. The evolutionary genetics of speciation.*in* A. E. Magurran and R. M. May, editors. Evolution of Biological Diversity. Oxford University Press, New York.

Craig, J. F. and J. M. Fletcher. 1981. Some results of fishing with survey gill nets in Windermere. Pages 236-244 Proceedings of the Second British Freshwater Fisheries Conference, University of Liverpool.

Crane, P. A., C. J. Lewis, E. J. Kretschmer, S. J. Miller, W. J. Spearman, A. L. DeCicco, M. J. Lisac, and J. K. Wenburg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus* Conservation Genetics **5**:737-741.

Cresko, W. A., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. A. Bell, C. B. Kimmel, and J. H. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback. Proceedings of the National Academy of Sciences **101**:6050-6055.

Danley, P. D. and T. D. Kocher. 2001. Speciation in rapidly diverging systems: lessons from Lake Malawi. Molecular Ecology **10**:1075-1086.

Danzmann, R. G., M. M. Ferguson, S. Skulason, S. S. Snorrason, and D. L. J. Noakes. 1991. Mitochondrial DNA diversity among four sympatric morphs of Arctic charr, *Salvelinus alpinus* L., from Thingvallavatn, Iceland Journal of Fish Biology **39**:649-659.

Day, T. and J. D. McPhail. 1996. The effect of behavioural and morphological plasticity on the foraging efficiency in the threespine stickleback (*Gasterosteus* sp.). Oecologia **108**:380-388.

Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. Evolution **48**:1723-1734.

Deines, P., J. Grey, H.-H. Richnow, and G. Eller. 2007. Linking larval chironomids to methane: seasonal variation of the microbial methane cycle and chironomid $\delta^{13}C$. Aquatic Microbial Ecology **46**:273-282.

DeNiro, M. J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**:495-506.

DeWitt, T. J., B. W. Robinson, and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology Evolutionary Ecology Research **2**:129-148.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution **13**:77-81.

DeWoody, J. A. and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology **56**:461-473.

Dobzhansky. 1937. Genetics and the origin of species. 1st edition. Columbia University Press, New York

Dobzhansky. 1951. Genetics and the origin of species. 3rd edition. Columbia University Press, New York

Dynes, J., P. Magnan, L. Bernatchez, and M. Rodriguez. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. Journal of Fish Biology **54**:955-972.

Elliott, J. M. and E. Baroudy. 1995. The ecology of Arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta*, in Windermere (northwest England). Nordic Journal of Freshwater Research **71**:33-48.

Evans, D. J. A., C. D. Clark, and W. A. Mitchell. 2005. The last British Ice Sheet: A review of the evidence utilised in the compilation of the glacial map of Britain. Earth-Science Reviews **70**:253-312.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783-791.

Felsenstein, J. 1993. PHYLIP (*Phylogeny Inference Package*), Version 3.5c. University of Washington, Seattle.

Fleming, I. A. and J. D. Reynolds. 2003. Salmonid breeding systems.*in* A. P. Hendry and S. C. Stearns, editors. Evolution Illuminated, Salmon and Their Relatives. Oxford University Press Oxford, USA.

Forseth, T., O. Ugedal, and B. Jonsson. 1994. The Energy Budget, Niche Shift, Reproduction and Growth in a Population of Arctic Charr, *Salvelinus alpinus.* Journal of Animal Ecology **63**:116-126.

Forseth, T., O. Ugedal, B. Jonsson, and I. A. Fleming. 2003. Selection on Arctic charr generated by competition from brown trout. Oikos **101.**

France, R. L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications Marine Ecology progress Series **124**:307-312.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Fraser, D., C. Adams, and F. A. Huntingford. 1998. Trophic polymorphism among Arctic charr *Salvelinus alpinus* L., from Loch Ericht, Scotland. Ecology of Freshwater Fish 7:184-191.

Fraser, D., F. A. Huntingford, and C. E. Adams. 2007. Foraging specialisms, prey size and life-history patterns: a test of predictions using sympatric polymorphic Arctic charr (*Salvelinus alpinus*). Ecology of Freshwater Fish.

Frost, W. E. 1965. Breeding Habits of Windermere Charr, *Salvelinus willughbii* (Gunther), and their Bearing on Speciation of these Fish. Proceedings of the Royal Society of London B **163**:232-284.

Frost, W. E. 1977. The food of Charr, *Salvelinus willughbii* (Gunther), in Windermere. Journal of Fish Biology **11**:531-547.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics **147**:915-925.

Gavrilets, S., A. Vose, M. Barluenga, W. Salzburger, and A. Meyer. 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. Molecular Ecology **16**:2893-2909.

Geffen, E., M. J. Anderson, and R. K. Wayne. 2004. Climate and habitat barriers to dispersal in the highly mobile grey wolf. Molecular Ecology **13**:2481-2490.

George, D., S. Maberly, and D. P. Hewitt. 2004. The influence of the North Atlantic Oscillation on the physical, chemical and biological characteristics of four lakes in the English Lake District. Freshwater Biology **49**:760-774.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology **21**:394-407.

Gillespie, G. J. and M. G. Fox. 2003. Morphological and life-history differentiation between littoral and pelagic forms of pumpkinseed. Journal of Fish Biology **62**:1099-1115.

Gislason, D., M. M. Ferguson, S. Skulason, and S. S. Snorrason. 1999. Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic charr (*Salvelinus alpinus*). Canadian Journal of Fisheries and Aquatic Science **56**:2229-2234.

Goodman, S. J. 1997. Rst Calc: a collection of computer programs for calculating estimates of genetic differentition from microsatellite data and a determining their significance. Molecular Ecology **6**:881-885.

Goudet, J. 2001. *FSTAT, A program to estimate and test gene diversities and fixation indices. Version 2.9.3.* . http://www.unil.ch/izea/softwares/fstat.html.

Gow, J. L., C. L. Peichel, and E. B. Taylor 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionary young species. Molecular Ecology **15**:739-752.

Grey, J. 2006. The use of stable isotope analyses in freshwater ecology: current awareness. Polish Journal of Ecology **54**:563-584.

Gudbjörg, Á. Ó., M. G. Ritchie, and S. S. Snorrason. 2006. Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. Biology letters **2**:250-252.

Guiguer, K. R. A., J. D. Reist, M. Power, and J. A. Babaluk. 2002. Using stable isotopes to confirm the trophic ecology of Arctic charr morphotypes from Lake Hazen, Nunavut, Canada. Journal of Fish Biology **60**:348-362.

Guo, S. W. and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics **48**:361-372.

Hartley, S. E., S. E. Bartlett, and W. S. Davidson. 1992. Mitochondrial DNA analysis of Scottish populations of Arctic charr, *Salvelinus alpinus* (L.). Journal of Fish Biology **40**:219-224.

Hatfield, T. 1997. Genetic Divergence in Adaptive Characters Between Sympatric Species of Stickleback. American Naturalist **149**:1009-1029.

Hatfield, T. and D. Schluter. 1999. Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. Evolution **53**:866-873.

Hegrenes, S. 2001. Diet-induced phenotypic plasticity of feeding morphology in the orangespotted sunfish, *Lepomis humilis*. Ecology of Freshwater Fish **10**:35-42.

Hendry, A. P. 2001. Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. Genetica **112-113**:515-534.

Hendry, A. P. and T. P. Quinn. 1997. Variation in adult life history and morphology among Lake Washington sockeye salmon (*Oncorhynchus nerka*) populations in relation to habitat features and ancestral affinities. Canadian Journal of Fisheries and Aquatic Sciences **54**:75-84.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid Evolution of Reproductive Isolation in the Wild: Evidence from Introduced Salmon. Science **290**:516-518.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society **58**:247-276.

Hillis, D. M., B. K. Mable, and C. Moritz. 1996. Applications of Molecular Systematics: the state of the field and a look to the future. Pages 515-543 *in* D. M. Hillis, C. Moritz, and B. K. Mable, editors. Molecular Systematics. Sinauer Associates, Massachusetts.

Hindar, K. and B. Jonsson. 1982a. Habitat and food segregation of dwarf and normal Arctic charr (*Salvelinus alpinus*) from Vangsvatnet Lake, Western Norway. Canadian Journal of Fisheries and Aquatic Sciences **39**:1030-1045.

Hindar, K. and B. Jonsson. 1993. Ecological polymorphism in Arctic charr. Biological Journal of the Linnean Society **48**:63-74.

Hindar, K., B. Jonsson, J. H. Andrew, and T. G. Northcote. 1988. Resource utilization of sympatric and experimentally allopatric cutthroat trout and Dolly Varden charr. Oecologia **74**:481-491.

Hindar, K. and K. Jonsson. 1982b. Habitat and food segregation of dwarf and normal Arctic charr (*Salvelinus alpinus*) from Vangsvatnet Lake, western Norway. . Canadian Journal of Fisheries and Aquatic Sciences **39**:1030-1045.

Ho, S. Y. W., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time Dependency of Molecular Rate Estimates and Systematic Overestimation of Recent Divergence Times. Molecular Biology and Evolution **22**:1561-1568.

Ho, S. Y. W., B. Shapiro, M. J. Philips, A. Cooper, and A. J. Drummond. 2007. Evidence for Time Dependency of Molecular Rate Estimates. Systematic Biology **56**:515-522.

Hoelzel, A. R. and A. Green. 1998. PCR protocols and population analysis by direct DNA sequencing and PCR-based DNA fingerprinting.*in* A. R. Hoelzel, editor. Molecular Genetic Analysis of Populations. Oxford University Press, Oxford.

Hori, M. 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish Science **260**:216-219.

Howard, D. J. 1993. Reinforcement: origins, dynamics and fate of an evolutionary hypothesis. Pages 46-69 *in* R. G. Harrison, editor. Hybrid zones and the evolutionary process. Oxford University Press, Oxford.

Hyslop, E. J. 1980. Stomach contents analysis-a review of methods and their application. Journal of Fish Biology **17**:411-429.

Igoe, F. and J. Hammar. 2004. The Arctic charr, *Salvelinus alpinus* (L.), species complex in Ireland: a secretive and threatened Ice age relict. Special Edition of Biology and the Environment, Royal Irish Academy **104**:73-92.

Igoe, F., M. O'Grady, C. Byrne, P. Gargan, W. Roche, and J. O'Neill. 2001. Evidence for the recent extinctions of two Arctic charr *Salvelinus alpinus* (L.) populations in the West of Ireland. Aquatic Conservation **11**:77-92.

Jastrebski, C. J. and B. W. Robinson. 2004. Natural selection and the evolution of replicated trophic polymorphisms in pumpkinseed sunfish (*Lepomis gibbosus*). Evolutionary Ecology Research **6**:285-305.

Jobling, M., E. H. Jørgensen, A. M. Arnesen, and E. Einar Ringø. 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential Aquaculture International **1**:20-46.

Johnson, L. 1980. The Arctic charr, *Salvelinus alpinus*. Pages 15-98 *in* E. K. Balon, editor. Charrs, Salmonid Fishes of the Genus Salvelinus. Junk, The Hague.

Johnson, T. C., C. A. Scholz, M. R. Talbot, K. Kelts, R. D. Ricketts, G. Ngobi, K. Beunig, I. Ssemmanda, and J. W. McGill. 1996. Lake Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. Science **273**.

Jones, I. D., I. J. Winfield, and F. Carse. 2008. Assessment of long-term changes in habitat availability for Arctic charr (*Salvelinus alpinus*) in a temperate lake using oxygen profiles and hydroacoustic surveys. Freshwater Biology **53**:393-402.

Jonsson, B. and N. Jonsson. 2001. Polymorphism and Speciation in Arctic charr. Journal of Fish Biology **58**:605-638.

Jorgensen, E. H. and M. Jobling. 1990. Feeding modes in Arctic charr, *Salvelinus alpinus* L.: the importance of bottom feeding for the maintenance of growth. Aquaculture **86**:379-385.

Kaufman, L. S., L. J. Chapman, and C. A. Chapman. 1997. Evolution in fast forward: haplochromine fishes of the Lake Victoria region. Endeavour **21**:23-30.

Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen, and R. I. Jones. 2006. A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. Journal of Applied Ecology **43**:1213-1222.

Kipling, C. 1972. The commercial fisheries of Windermere. Transactions of the Cumberland Westmorland Antiquarian Archaeological Society **72**:156-204.

Kipling, C. and E. D. Le Cren. 1984. Mark-recapture experiments on fish in Windermere, 1943–1982. Journal of Fish Biology **24**:395-414.

Kircheis, F. W. 1989. History of landlocked Arctic char management in Maine, USA. Physiology and Ecology Japan **1**:615-623.

Klemetsen, A., P.-A. Amundsen, J. B. Dempson, B. Jonsson, N. Jonsson, M. F. O'Connell, and E. Mortensen. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecology of Freshwater Fish **12**:1-59.

Klemetsen, A., P.-A. Amundsen, R. Knudsen, and B. Hermansen. 1997. A profundal, winter spawning morph of Arctic charr, *Salvelinus alpinus* (L.) in lake Fjellfrosvatn, northern Norway. Nordic Journal of Freshwater Research **73**:13-23.

Klemetsen, A., J. Elliott, R. Knudsen, and P. Sorensen. 2002. Evidence for genetic differences in the offspring of two sympatric morphs of Arctic charr. Journal of Fish Biology **60**:933-950.

Klemetsen, A. and P. E. Grotnes. 1975. Food and habitat segregation by two sympatric Arctic charr populations. Verh. Internat. Verein. Limnol. **19**:2521-2528.

Knight, M. E. and G. F. Turner. 2004. Laboratory mating trials indicate incipient speciation by sexual selectionamong populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. Proceedings of the Royal Society B **271**:675-680.

Knudsen, R., A. Klemetsen, P.-A. Amundsen, and B. Hermansen. 2006. Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake. Proceedings of the royal society B **273**:2291-2298.

Knudsen, R., R. Kristoffersen, and P.-A. Amundsen. 1997. Parasite communities in two sympatric morphs of Arctic charr, *Salvelinus alpinus* (L.), in northern Norway. Canadian Journal of Zoology **75**:2003-2009.

Kocher, T. D. 2004. Adaptive evolution and explosive speciation: the cichid fish model. Nature Reviews **5**:288-298.

Koskinen, M. T., J. Piironen, and C. R. Primmer. 2001. Interpopulation genetic divergence in European grayling (*Thymallus thymallus*, Salmonidae) at a microgeographic scale: implications for conservation. Conservation Genetics **2**.

Kottelat, M. and J. Freyhof. 2007. Handbook of European Freshwater Fishes. Publications Kottelat, Cornol, Switzerland.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment Briefings in Bioinformatics **5**:150-163.

Langeland, A., J. H. L'Abee-Lund, B. Jonsson, and N. Jonsson. 1991. Resource partitioning and niche shift in Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta*. Journal of Animal Ecology **60**:895-912.

Langeland, A. and T. Nost. 1994. Introduction of roach (*Rutilus rutilus*) in an oligohumic lake: 1. Competition impacts on whitefish (*Coregonus lavaretus*). Verh. Internat. Verein. Limnol. **25**:2113-2117.

Langeland, A. and T. Nost. 1995. Gill raker structure and selective predation on zooplankton by particulate feeding fish. Journal of Fish Biology **47**:719-732.

Langella, O. 2001. *Populations* (version 1.2.24) population genetic software (individuals or population distances, phylogenetic trees).

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2004. Clustal W and Clustal X version 2.0. Bioinformatics **23**:2947-2948.

Larson, R. J. 1980. Competition, Habitat Selection, and the Bathymetric Segregation of Two Rockfish (*Sebastes*) Species. Ecological Monographs **50**:221-239.

Larsson, S., T. Forseth, I. Berglund, A. J. Jensen, I. Naslund, J. M. Elliott, and B. Jonsson. 2005. Thermal adaptation of Arctic charr: experimental studies of growth in eleven charr populations from Sweden, Norway and Britain. Freshwater Biology **50**:353-368.

Le Cren, E. D. and C. Kipling. 1963. Some marking experiments on spawning populations of charr. Special Publication for International Commision for the Northwest Atlantic Fisheries **4**:130-139.

Logan, J., H. Haas, L. Deegan, and E. Gaines. 2006. Turnover rates of nitrogen stable isotope in the saltmarsh mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. Oecologia **147**:391-395.

Losos, J. B., D. A. Creer, D. Glossip, R. Goellner, A. Hampton, G. Roberts, N. Haskell, P. Taylor, and J. Ettling. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution **54**:301-305.

Lu, G. and L. Bernatchez. 1999. Correlated trophic specialisation and genetic divergence oin sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution **33**:1491-1505.

Maberly, S., L. King, C. E. Gibson, L. May, R. I. Jones, M. M. Dent, and C. Jordan. 2003. Linking nutrient limitation and water chemistry in upland lakes to catchment characteristics Hydrobiologia **506**:93-91.

MacKenzie, A., A. S. Ball, and S. R. Virdee. 1998. Instant Notes in Ecology. BIOS Scientific Publishers Limited, Oxford.

Maekawa, K., S. Nakano, and S. Yamamoto. 1994. Spawning behaviour and size-assortative mating of Japanese charr in an artificial lake-inlet stream system. Environmental Biology of Fishes **39**:109-117.

Magurran, A. E. 1999. Population differentiation without speciation.*in* A. E. Magurran and R. M. May, editors. Evolution of Biological Diversity. Oxford University Press, New York.

Maitland, P. S. 1992. The status of Arctic charr, *Salvelinus alpinus* (L.), in southern Scotland: a cause for concern. Freshwater Forum **2**:212-227.

Maitland, P. S. 1995. World status and conservation of the Arctic charr *Salvelinus alpinus* (L.). Nordic Journal of Freshwater Research **71**:113-127.

Maitland, P. S., I. J. Winfield, I. D. McCarthy, and F. Igoe. 2007. The status of Arctic charr *Salvelinus alpinus* in Britain and Ireland. Ecology of Freshwater Fish **16**:6-19.

Malmquist, H. J. 1992. Phenotype-specific feeding behaviour of two Arctic charr *Salvelinus alpinus* morphs. Oecologia **92**:354-361.

Malmquist, H. J., S. S. Snorrason, S. Skulason, B. Jonsson, O. T. Sandlund, and P. M. Jonasson. 1992. Diet Differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. Journal of Animal Ecology **61**:21-35.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

McArdle, B. H. and M. J. Anderson. 2001. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. Ecology **82**:290-297.

McCarthy, I. D., D. Fraser, S. Waldron, and C. E. Adams. 2004. A stable isotope analysis of trophic polymorphism among Arctic charr from Loch Ericht, Scotland. Journal of Fish Biology **65**:1435-1440.

McCubbing, D. J. F., B. D. Bayliss, and V. M. Locke. 1998. Spawning migration of radio tagged landlocked Arctic Charr, *Salvelinus alpinus* L. in Ennerdale Lake, the English Lake District. Hydrobiologia **371/372**:173-180.

McDowall, R. M. 2001. Anadromy and homing: two life-history traits with adaptive synergies in salmonid fishes? Fish and Fisheries **2**:78-85.

McPhail, J. D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): Evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. Canadian Journal of Zoology **70**:361-369.

Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution **41**:1357-1369.

Meyer, A. 1993. Phylogenetic relationships and evolutionary processes in East African cichlid fishes. Trends in Ecology and Evolution **8**:279-284.

Mikheev, V. N., C. E. Adams, F. A. Huntingford, and J. E. Thorpe. 1996. Behavioural responses of benthic and pelagic Arctic charr to substratum heterogeneity. Journal of Fish Biology **49**:494-500.

Mills, C. A. 1989. The Windermere populations of Arctic charr, *Salvelinus alpinus*. Physiology and Ecology Japan Special **1**:371-382.

Mills, C. A., S. I. Heaney, C. Butterwick, J. E. Corry, and J. M. Elliott. 1990. Lake enrichment and the status of Windermere charr, *Salvelinus alpinus* (L.). Journal of Fish Biology **37**:167-174.

Mills, C. A. and M. A. Hurley. 1990. Long-term studies on the Windermere populations of perch (*Perca fluviatilis*), pike (*Exos lucius*) and Arctic charr (*Salvelinus alpinus*). Freshwater Biology **23**:119-136.

Mills, L. S. and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology **10**:1509-1518.

Minagawa, M. and E. Wada. 1984. Stepwise enrichment of 15 N along food chains: Further evidence and the relation between 15 N and animal age. Geochimica et Cosmochimica Acta **48**:1135-1140.

Mittelbach, G. G. 1981. Foraging Efficiency and Body Size: A Study of Optimal Diet and Habitat Use by Bluegills. Ecology **62**:1370-1386.

Mittelbach, G. G., C. W. Osenburg, and P. C. Wainwright. 1999. Variation in feeding morphology between pumpkinseed populations: Phenotypic plasticity or evolution? Evolutionary Ecology Research **1**:111-128.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends in Ecology and Evolution **9**:373-375.

Næsje, T. F., J. A. Vuorinen, and O. T. Sandlund. 2004. Genetic and morphometric differentiation among sympatric spawning stocks of whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. Joural of Limnology **63**:233-243.

Neff, B. D. 2004. Stabilising selection on genomic divergence in a wild fish population. Proceedings of the National Academy of Sciences **101**:2381-2385.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nei, M. and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics **105**:207-217.

Neville, H. M., D. J. Isaak, J. B. Dunham, R. F. Thurow, and B. E. Rieman. 2006. Fine-scale natal homing and localised movement as shaped by sex and spawning habitat in Chinook salmon: insights from spatial autocorrelation analysis of individual genotypes. Molecular Ecology **15**:4589-4602.

Nielsen, R. and J. Wakely. 2001. Distinguishing Migration From Isolation: A Markov Chain Monte Carlo Approach. Genetics **158**:885-896.

Nilsson, N.-A. 1963. Interaction Between Trout and Char in Scandinavia. Transactions of the American Fisheries Society **92**:276-285.

Noor, M. A. F. 1999. Reinforcement and other consequences of sympatry. Heredity **83**:503-508.

Nordeng, H. 1961. On the Biology of charr (*Salmo alpinus* L.) in Salangen, North Norway. I. Age and spawning frequency determined from scales and otoliths. Nytt Mag. Zool. **10**:67-123.

Nordeng, H. 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Canadian Journal of Fisheries and Aquatic Sciences **40**:1372-1387.

Nussey, D. H., A. J. Wilson, and J. E. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology **20**:831-844.

Orr, M. R. and T. B. Smith. 1998. Ecology and Speciation. Trends in Ecology and Evolution **13**:502-506.

Ostbye, K., T. F. Næsje, L. Bernatchez, O. T. Sandlund, and K. Hindar. 2005. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. Journal of Evolutionary Biology **18**:683-702.

Parker, J. E. and S. C. Maberly. 2000. Biological response to lake remediation by phosphate stripping: control of *Cladophora*. Freshwater Biology **44**:303-309.

Parsons, K. J. and B. W. Robinson. 2006. Replicated evolution of integrated plastic responses during early adaptive divergence. Evolution **60**:801-813.

Parsons, K. J. and B. W. Robinson. 2007. Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favours resource polymorphism. Journal of Evolutionary Biology **20**:673-684.

Partington, J. D. and C. A. Mills. 1988. An electrophoretic and biometric study of Arctic charr, *Salvelinus alpinus* (L.), from ten British lakes. Journal of Fish Biology **33**:791-814.

Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. E. Cole, P. F. Colosimo, C. A. Buerkle, D. Schluter, and D. M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature **414**:901-905.

Persson, L. 1987. Competition-induced switch in young of the year perch, *Perca fluviatilis*: an experimental test of resource limitation. Environmental Biology of Fishes **19**:235-239.

Peterson, B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**:293-320.

Pfennig, D. W. 1992. Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. Evolution **46**:1408-1420.

Pfennig, D. W., A. M. Rice, and R. A. Martin. 2007. Field and experimental evidence for competition's role in phenotypic divergence. Evolution **61**:257-271.

Pickering, A. D. and D. W. Sutcliffe. 2001. Windermere: Restoring the Health of England's Largest Lake. Freshwater Biological Association, Ambleside.

Pigluicci, M. and C. J. Murren. 2003. Perspective: Genetic assimilation and a possible evolutionary paradox: Can Macroevolution sometimes be so fast as to pass us by? Evolution **57**:1455-1464.

Pinnegar, J. K. and N. V. C. Polunin. 1999. Differential fractionation of δ13C and δ15N among fish tissues: implications for the study of trophic interactions. Functional Ecology **13**:225-231.

Price, T. D., A. Qvarnstrom, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society B **270**:1433-1440.

Primmer, C. R., T. Aho, J. Piironen, A. Estoup, J.-M. Cornuet, and E. Ranta. 1991. Microsatellite Analysis of Hatchery Stocks and Natural Populations of Arctic Charr, *Salvelinus Alpinus*, from the Nordic Region: Implications for Conservation. Hereditas **130**.

Primmer, C. R., A. J. Veselov, A. Zubchenko, A. Poututkin, I. Bakhmet, and M. T. Koskinen. 2006. Isolation by distance within a river system: genetic

population structuring of Atlantic salmon, *Salmo salar*, in tributaries of the Varzuga River in northwest Russia. Molecular Ecology **15**:653-666.

Pritchard, J. R. and D. Schluter. 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. Evolutionary Ecology Research **3**:209-220.

Quinn, T. P. and A. H. Dittman. 1990. Pacific salmon migrations and homing: mechanisms and adaptive significance. Trends in Ecology and Evolution **5**:174-177.

Ramsbottom, A. E. 1976. Depth Charts of the Cumbrian Lakes. Freshwater Biological Association, Ambleside.

Raymond, M. and F. Rousset. 2001. *GENEPOP. Version 3.3: Population Genetics Software for exact tests and ecumenicism.*

Reist, J. D., E. Gyselman, J. A. Babaluk, J. J. D, and R. Wissinik. 1995. Evidence of two morphotypes of Arctic char (*Salvelinus alpinus* (L.) from Lake Hazen, Ellesmere Island, Northwest Territories, Canada. Nordic Journal of Freshwater Research **71**:396-410.

Reist, J. D., F. J. Wrona, T. D. Prowse, M. Power, J. B. Dempson, J. R. King, and R. J. Beamish. 2006. An Overview of Effects of Climate Change on Selected Arctic Freshwater and Anadromous Fishes. Ambio **35**:381-387.

Reznick, D. N. and C. K. Ghalambor. 2005. Selection in nature: Experimental manipulations of natural populations. Integrative and Comparitive **45**:456-462.

Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the Rate of Evolution in Natural Populations of Guppies (*Poecilia reticulata*). Science **275**:1934-1937.

Rice, A. M. and D. W. Pfennig. 2007. Character displacement: *in situ* evolution of novel phenotypes or sorting of pre-existing variation? Journal of Evolutionary Biology **20**:448-459.

Rice, W. R. and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution **47**:1637-1650.

Riget, F., E. Jeppesen, F. Landkildehus, T. L. Lauridsen, P. Geertz-Hansen, K. Christofferson, and H. Sparholt. 2000. Landlocked Arctic charr (*Salvelinus alpinus*) population structure and lake morphometry in Greenland - is there a connection? Polar Biology **23**:550-558.

Rios-Cardenas, O. and M. S. Webster. 2008. A molecular genetic examination of the mating system of pumpkinseed sunfish reveals high pay-offs for specialized sneakers. Molecular Ecology **17**:2310-2320.

Robinson, B. W. and D. S. Wilson. 1994. Character Release and Displacement in Fishes: A Neglected Literature. American Naturalist **144**:596.

Robinson, B. W. and D. S. Wilson. 1995. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evolutionary Ecology **10**:631-652.

Robinson, B. W. and D. S. Wilson. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evolutionary Ecology **10**:631-652.

Robinson, B. W., D. S. Wilson, and A. S. Margosian. 2000. A pluralistic analysis of character release in pumkinseed sunfish (*Lepomis gibbosus*). Ecology **81**:2799-2812.

Rogers, A. R. and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic distances. Molecular Biology and Evolution **9**:552-569.

Rousset, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics **142**:1357-1362.

Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coelescent and other methods. Bioinformatics **19**:2496-2497.

Rundle, H. D. and D. Schluter. 1998. Reinforcement of stickleback mating preferences: Sympatry breeds contempt. Evolution **52**:200-208.

Sandlund, O. T., K. Gunnarsson, P. M. Jonasson, B. Jonsson, T. Lindem, K. P. Magnusson, H. J. Malmquist, H. Sigurjonsdottir, S. Skulason, and S. S. Snorrason. 1992. The Arctic Charr Salvelinus alpinus in Thingvallavatn. Oikos **64**:305-351.

Schliewen, U. K., D. Tautz, and S. Paabo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature **368**:629-632.

Schlotterer, C. 1998. Microsatellites.*in* A. R. Hoelzel, editor. Molecular Genetic Analysis of Populations. Oxford University Press, Oxford.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science **266**:798-801.

Schluter, D. 1995. Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. Ecology **76**:82-90.

Schluter, D. 1996. Ecological speciation in postglacial fishes. Philosophical Transactions of the Royal Society, London, Series B **351**:807-814.

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford University press, Oxford.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology and Evolution **16**:372-380.

Schluter, D. and J. D. McPhail. 1992. Ecological Character Displacement and Speciation in Sticklebacks. American Naturalist **140**:85.

Schluter, D. and J. D. McPhail. 1993. Character displacement and replicate adaptive radiation. Trends in Ecology and Evolution **8**:197-200.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetics analysis. Vers. 2.000 Genetics and Biometry Lab., Dept. of Anthropology, Univ. of Geneva.

She, J. X., M. Autem, G. Kotulas, N. Pasteur, and F. Bonhomme. 1987. Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis (*Teleosts, Soleidae). Biological Journal of the Linnean Society **32**:357-371.

Simpson, G. G. 1953. The major features of evolution. Columbia University Press, New York.

Skulason, S. and T. B. Smith. 1995. Resource Polymorphisms in Vertebrates. TREE **10**:366-371.

Skulason, S., S. S. Snorrason, and B. Jonsson. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr. Pages 70-92 *in* A. E. Magurran and R. M. May, editors. Evolution of Biological Diversity. Oxford University Press, Oxford.

Skulason, S., S. S. Snorrason, D. L. G. Noakes, and M. M. Ferguson. 1996. Genetic basis of life history variations among sympatric morphs of Arctic charr (*Salvelinus alpinus*). Canadian Journal of Fisheries and Aquat Sciences **53**:1807-1813.

Skulason, S., S. S. Snorrason, D. L. G. Noakes, M. M. Ferguson, and H. J. Malmquist. 1989. Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. Journal of Fish Biology **1989**:225-232.

Smith, T. B. 1993. Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*. Nature **363**:618-620.

Smith, T. B. and S. Skulason. 1996. The evolutionary significance of resource polymorphisms in fishes, amphibians and birds. Annual Review of Ecology and Systematics **27**:111-133.

Snorrason, S. S., S. Skulason, B. Jonsson, H. J. Malmquist, P. M. Jonasson, O. T. Sandlund, and T. Lindem. 1994. Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): Morphological divergence and ontogenetic niche shifts. Biological Journal of the Linnean Society **52**:1-18.

Sparks, T. H., N. Collinson, H. Crick, P. Croxton, M. Edwards, K. Huber, D. Jenkins, D. Johns, F. Last, S. Maberly, M. Marquiss, J. Pickup, D. Roy, D. Sims, D. Shaw, A. Turner, A. Watson, I. Woiwod, and K. Woodbridge. 2006. Natural Heritage Trends of Scotland: phenological indicators of climate change.

Stewart, I. J., S. M. Carlson, C. P. Boatright, G. B. Buck, and T. P. Quinn. 2004. Evidence for fine-scale natal homing among island beachspawning sockeye salmon, *Oncorhynchus nerka*. Ecology of Freshwater Fish **67**:104-111.

Sturmbauer, C. and A. Meyer. 1992. Genetic divergence,speciation and morphological status in a lineage of African cichlid fishes. Nature **358**:578-581.

Sutcliffe, D. W. and T. R. Carrick. 1983. Chemical composition of water-bodies in the English Lake District: relationships between chloride and other major ions related to solid geology, and a tentative budget for Windermere. Freshwater Biology **13**:323-352.

Svennig, M.-A., A. Klemetsen, and T. Olsen. 2007. Habitat and food choice of Arctic charr in Linnevatn on Spitsbergen, Svalbard: the first year-round investigation in a High Arctic lake. Ecology of Freshwater Fish **16**:70-77.

Swofford, D. L. 2000. PAUP*. Phylogenetic analysis using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Syvaranta, J., S. Vesala, M. Rask, J. Ruvhijarvi, and R. I. Jones. 2008. Evaluating the utility of stable isotope analyses of archived freshwater sample material. Hydrobiologia **600**:121-130.

Taggart, J. B., I. S. McLaren, D. W. Hay, J. H. Webb, and A. F. Youngson. 2001. Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. Molecular Ecology **10**:1047-1060.

Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics **123**:597-601.

Taylor, E. B. 1999. Species pairs of north temperate freshwater fishes: Evolution, taxonomy, and conservation Reviews in Fish Biology and Fisheries **9**:299-324.

Taylor, E. B. and P. Bentzen. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America Evolution **47**:813-832.

Taylor, E. B. and J. D. McPhail. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. Biological Journal of the Linnean Society **66**:271-291.

Turner, G. F. 1999. Explosive speciation of African cichlid fishes. Pages 113-129 *in* A. E. Magurran and R. M. May, editors. Evolution of Biological Diversity. Oxford University Press, Oxford.

Turner, G. F. 2007. Adaptive radiation of cichlid fish. Current Biology **17**:r827-r831.

Turner, G. F., O. Seehausen, M. E. Knight, C. J. Allender, and R. L. Robinson. 2001. How many species of cichlid fishes are there in African lakes? Molecular Ecology **10**:793-806.

Van Oosterhut, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**:535-538.

Van Oppen, M. J. H., G. F. Turner, C. Rico, J. C. Deutsch, K. M. Ibrahim, R. L. Robinson, and G. M. Hewitt. 1997. Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fish Proceedings of the royal society B **264**:1803-1812.

Vander Zanden, M. J. and J. B. Rasmussen. 1999. Primary Consumer $\delta^{13}C$ and $\delta^{15}N$ and the Trophic Position of Aquatic Consumers. Ecology **80**:1395-1404.

Vines, T. H. and D. Schluter. 2006. Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. Proceedings of the royal society B **273**:911-916.

Waddington, C. H. 1961. Genetic assimilation. Advances in Genetics **10**:257-293.

Wahlund, S. 1928. The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. Hereditas **11**:65-106.

Wainright, S. C., M. J. Fogarty, R. C. Greenfield, and B. Fry. 1993. Long term changes in the Georges Bank food web: trends in stable isotope composition of fish scales Marine Biology **115**:481-493.

Walker, A. F. and R. B. Greer. 1988. Two ecologically distinct forms of Arctic charr *Salvelinus alpinus* (L.) in Loch Rannoch, Scotland. Biological Conservation **43**:43-61.

Wandeler, P., S. Smith, P. A. Morin, R. A. Pettifor, and S. M. Funk. 2003. Patterns of nuclear DNA degeneration over time — a case study in historic teeth samples. Molecular Ecology **12**:1087-1093.

Webb, P. W. 1984. Body Form, Locomotion and Foraging in Aquatic Vertebrates. American Zoologist **24**:107-120.

Weir, B. S. and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution **38**:1358-1370.

Werner, E. E. and D. J. Hall. 1976. Niche shifts in sunfishes: experimental evidence and significance. Science **191**:404-406.

West-Eberhard, M. J. 1989. Phenotypic plastcity and the origins of diversity. Annual Review of Ecology and Systematics **20**:249-278.

West-Eberhard, M. J. 2003. Developmental Plasticity and the Evolution. Oxford University Press, New York.

Westgaard, J. I., A. Klemetsen, and R. Knudsen. 2004. Genetic differences between two sympatric morphs of Arctic charr confirmed by microsatellite DNA. Journal of Fish Biology **65**:1185-1191.

Willoughby, L. G. 1976. Freshwater Biology. Hutchinson & Co., London.

Wilson, A. J., D. Gíslason, S. Skúlason, S. S. Snorrason, C. E. Adams, G. Alexander, R. G. Danzmann, and M. M. Ferguson. 2004. Population genetic structure of Arctic Charr, *Salvelinus alpinus* from northwest Europe on large and small spatial scales. Molecular Ecology **13**:1129-1142.

Wilson, C. C., P. D. N. Hebert, J. D. Reist, and J. B. Dempson. 1996. Phylogeography and postglacial dispersal of Arctic charr *Salvelinus alpinus* in North America. Molecular Ecology **5**.

Winfield, I. J. 1992. Threats to the lake fish communities of the U.K. arising from eutrophication and species introductions. Netherlands Journal of Zoology **42**:233-242.

Winfield, I. J. 2004. Fish in the littoral zone: ecology, threats and management. Limnologica **34**:124-131.

Winfield, I. J. and N. C. Durie. 2004. Fish introductions and their management in the English Lake District. Fisheries Management and Ecology **11**:1-7.

Winfield, I. J., J. M. Fletcher, D. P. Hewitt, and J. B. James. 2004a. Long-term trends in the timing of the spawning season of Eurasion perch (*Perca fluviatilis*) in the north basin of Windermere, UK. Pages 95-96 *in* Proceedings of Percis III: The Third International Percid Fish Symposium, University of Wisconsin Sea Grant Institute, Madison, WI.

Winfield, I. J., J. M. Fletcher, and B. J. James. 2004b. Conservation Ecology of the Vendace (*Coregonus albula*) in Bassenthwaite Lake and Derwent Water, UK. Annales Zoologici Fennici **41**:155-164.

Winfield, I. J., J. M. Fletcher, and B. J. James. 2005. Review and assessment of brown trout and Arctic charr stocks in Windermere., Report to Environment Agency, North West Region.

Winfield, I. J., J. M. Fletcher, and B. J. James. 2006. Fish survey data from three Cumbrian lakes. Report to Environment Agency, North West Region. Centre for Ecology and Hydrology, Lancaster.

Winfield, I. J., J. M. Fletcher, and B. J. James. 2007. The Arctic charr (*Salvelinus alpinus*) populations of Windermere, UK: population trends associated with eutrophication, climate change and increased abundance of roach (*Rutilus rutilus*). Environmental Biology of Fishes **83**:25-35.

Winfield, I. J., J. B. James, and J. M. Fletcher. 2004c. Assessment of the fish community of Coniston Water. Report to Environment Agency, North West Region. Centre for Ecology and Hydrology Lancaster.

Wood, C. C. and C. J. Foote. 1996. Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). Evolution **50**:1265-1279.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution **19**:395-420.

Wright, S. 1982. The shifting balance theory and macroevolution. Annual Review of Genetics **16**:1-20.

APPENDIX 1: Hardy-Weinburg results

Windermere

Locus	Ho	He	P value
Smm17	0.79	0.76	0.99
Smm10	0.73	0.66	0.15
Ssa85	0.79	0.9	0
Omm1302	0.8	0.75	0
Sal J81	0.74	0.71	0.18
Omm1337	0.78	0.75	0.2
Sfo334	0.68	0.91	0
Mst85	0.72	0.77	0
SalO23	0.86	0.93	0
SalD30	0.9	0.92	0

Coniston Water

Locus	Ho	He	P value
Smm17	0.45	0.56	0
Smm10	0.21	0.32	0
Ssa85	0.8	0.82	0
Omm1302	0.66	0.6	0.42
Sal J81	0.62	0.55	0.07
Omm1337	0.87	0.72	0
Sfo334	0.75	0.79	0.09
Mst85	0.79	0.84	0
SalO23	0.72	0.88	0
SalD30	0.52	0.78	0

Buttermere

Locus	Ho	He	P value
Smm17	0.67	0.59	0.5
Smm10	0.58	0.43	0.49
Ssa85	0.33	0.59	0
Omm1302	0.58	0.49	0.58
Sal J81	0.5	0.6	1
Omm1337	0.5	0.55	0.7
Sfo334	0.67	0.83	0.3
Mst85	-	-	-
SalO23	0.25	0.66	0
SalD30	0.75	0.8	0.3

Ennerdale

Locus	Ho	He	P value
Smm17	0.43	0.71	0.25
Smm10	0.57	0.79	0.03
Ssa85	0.43	0.74	0.15
Omm1302	0.57	0.56	1
Sal J81	0.71	0.54	1
Omm1337	1	0.7	0.14
Sfo334	0.57	0.92	0.19
Mst85			
SalO23	0.86	0.86	0.5
SalD30	0.57	0.85	0.06

Haweswater

Locus	Ho	He	P value
Smm17	0.25	0.44	0.21
Smm10	0.5	0.68	0.41
Ssa85	0.63	0.84	0.19
Omm1302	0.86	0.69	0.26
Sal J81	0.75	0.71	0.41
Omm1337	1	0.82	0.03
Sfo334	0.88	0.92	0.72
Mst85	0.75	0.82	0.55
SalO23	0.75	1	0.12
SalD30	0.71	0.74	1

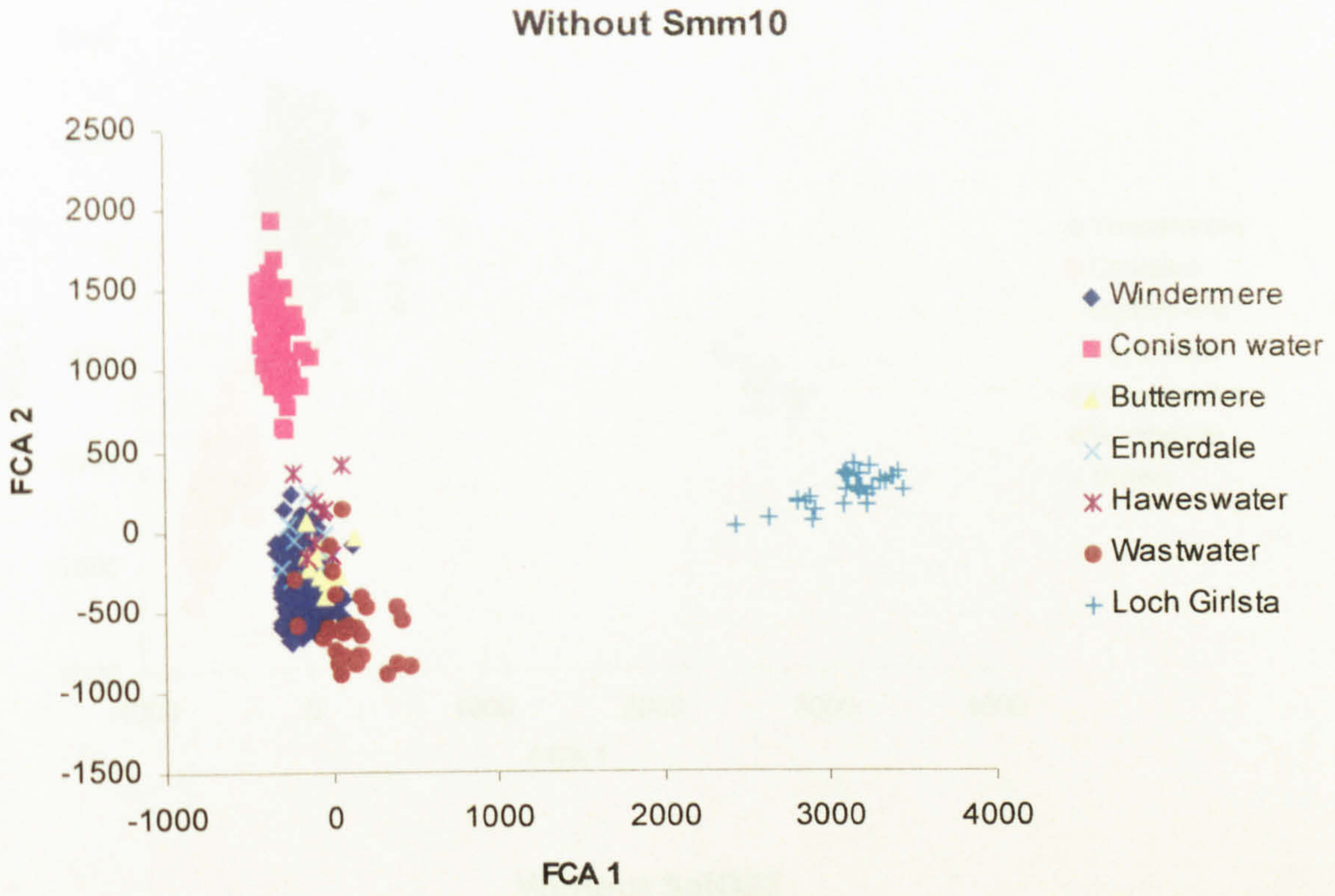
Wastwater

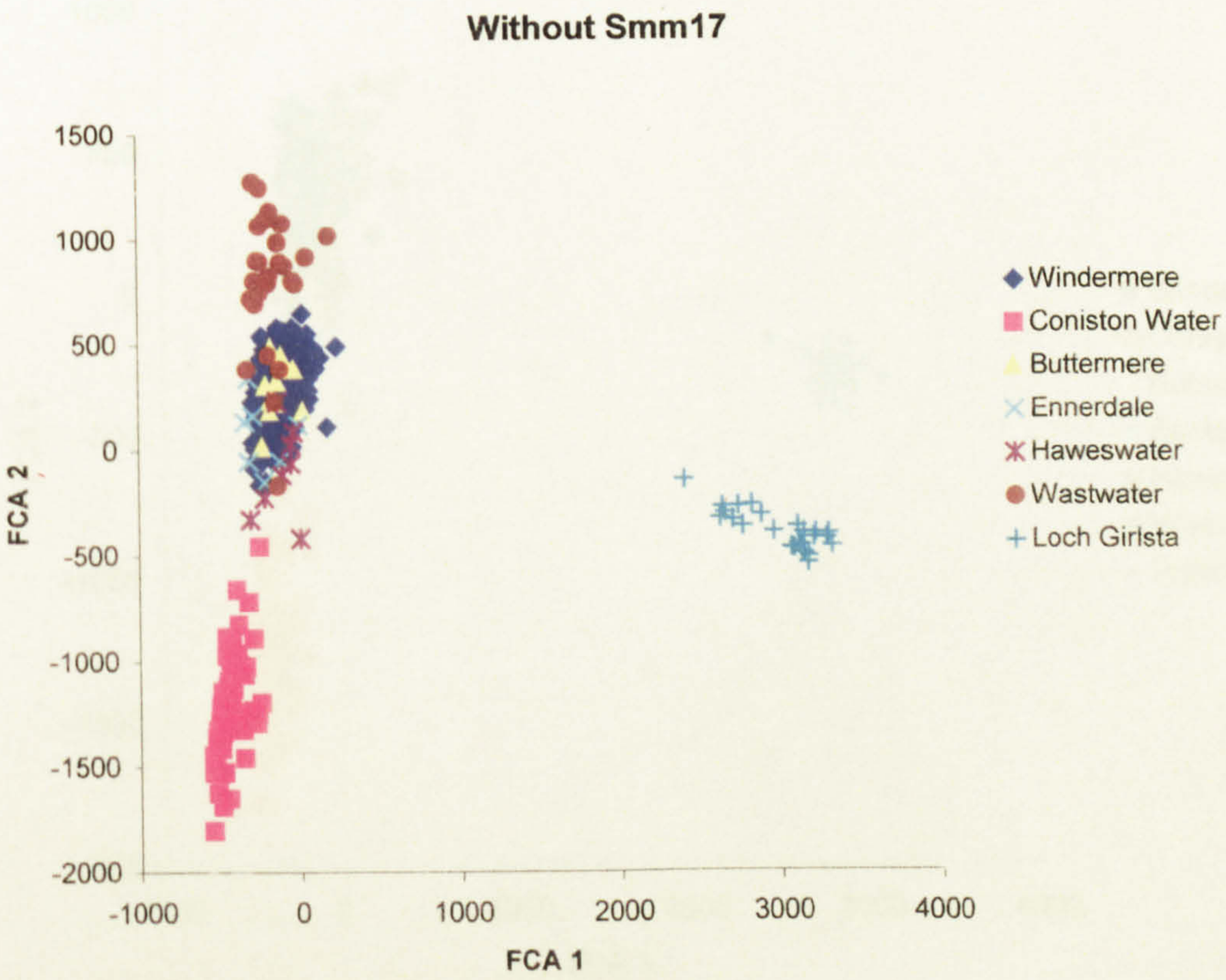
Locus	Ho	He	P value
Smm17	0.71	0.75	0.24
Smm10	0.67	0.7	0.08
Ssa85	0.75	0.84	0
Omm1302	0.16	0.19	1
Sal J81	0.48	0.72	0
Omm1337	0.68	0.61	0.34
Sfo334	0.67	0.92	0
Mst85	1	0.83	1
SalO23	1	0.93	1
SalD30	0.87	0.91	0.49

Loch Girlsta

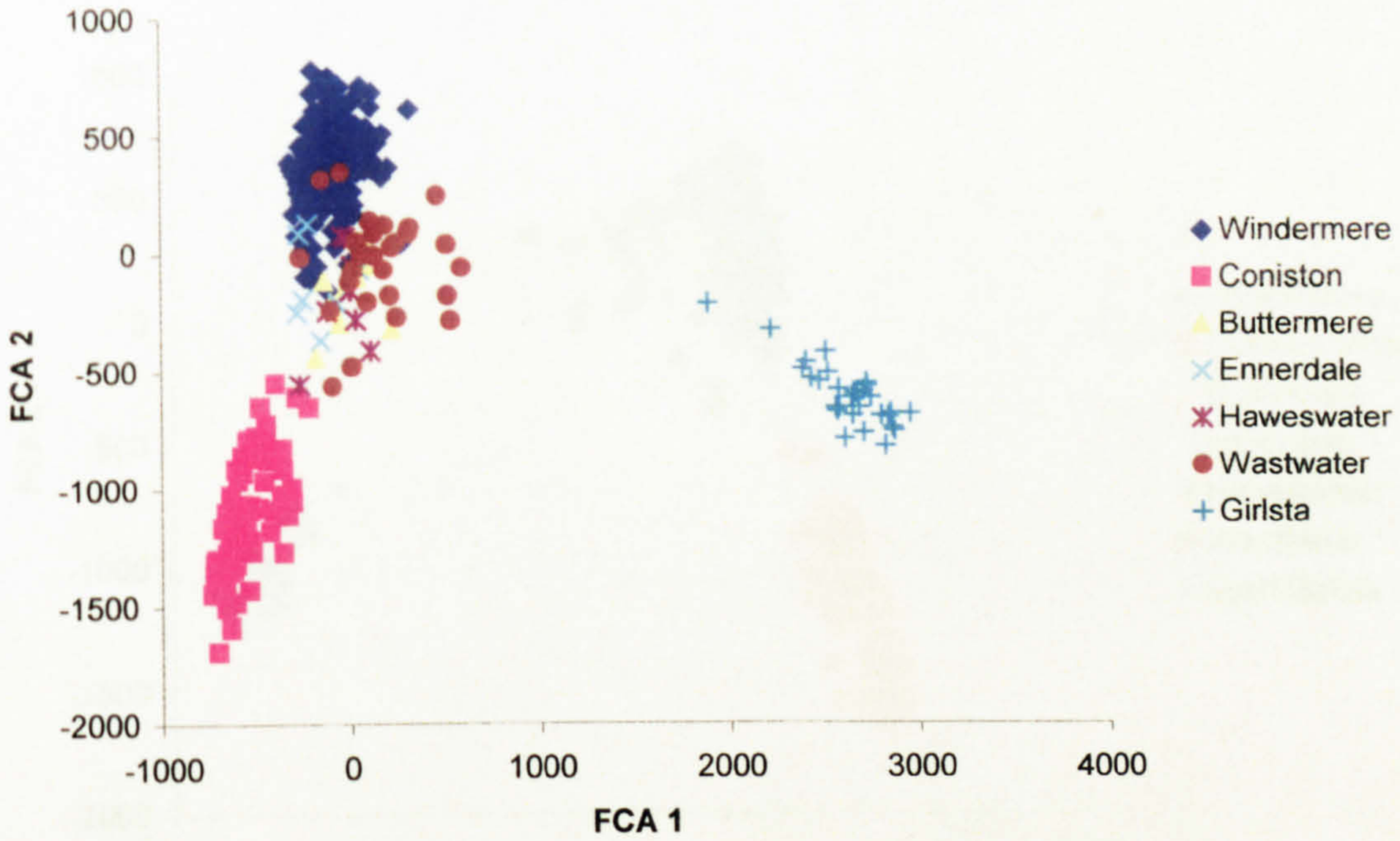
Locus	Ho	He	P value
Smm17	0.77	0.71	0.55
Smm10	0.6	0.44	0.09
Ssa85	0.77	0.78	0
Omm1302	0.83	0.64	0.22
Sal J81	0.7	0.65	0.57
Omm1337	0.79	0.68	0.01
Sfo334	0.6	0.46	0.26
Mst85	-	-	-
SalO23	0.56	0.75	0.3
SalD30	-	-	-

APPENDIX 2: Factorial Correspondence Analysis of nine microsatellite with the removal of one locus at a time

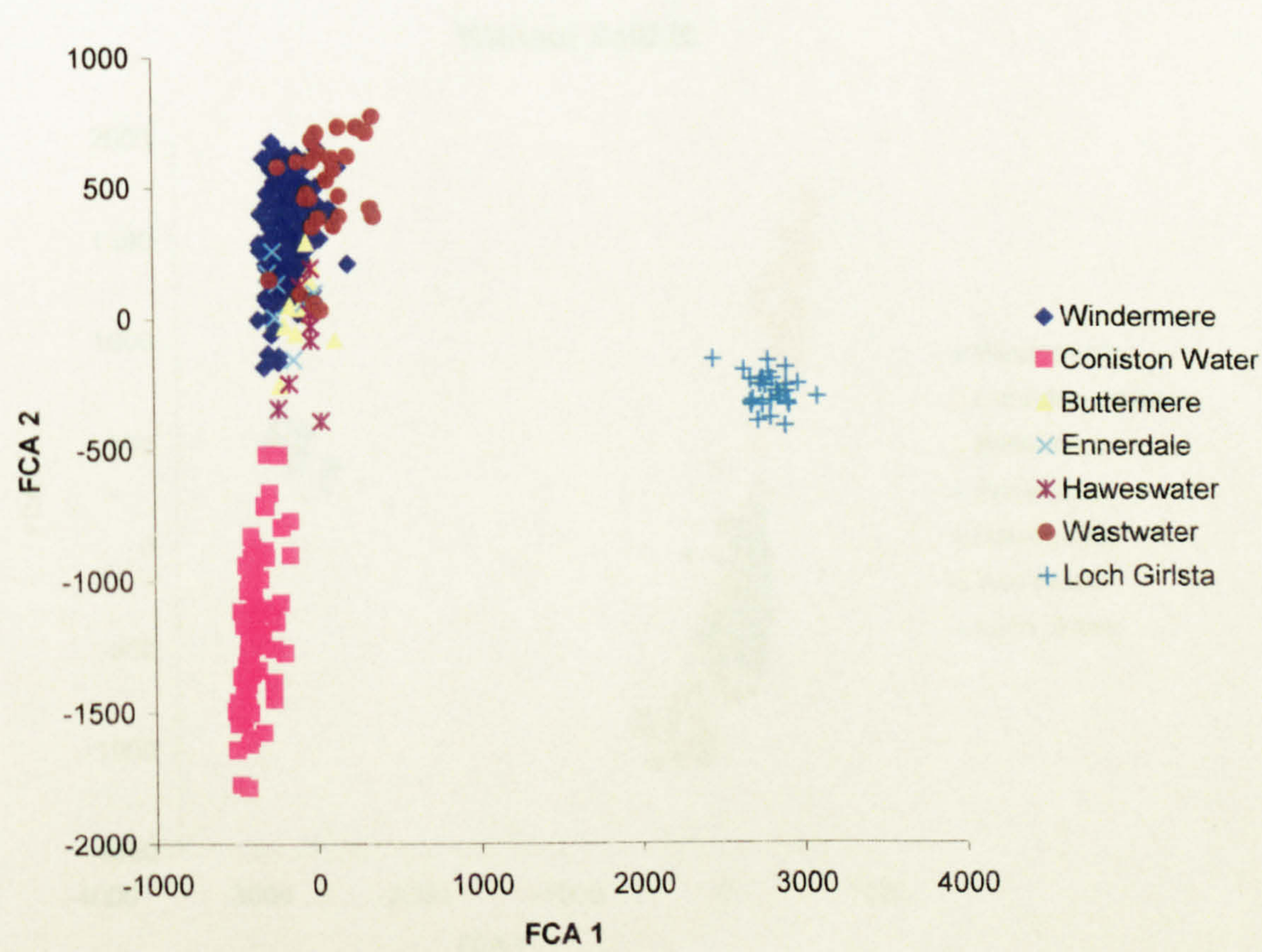




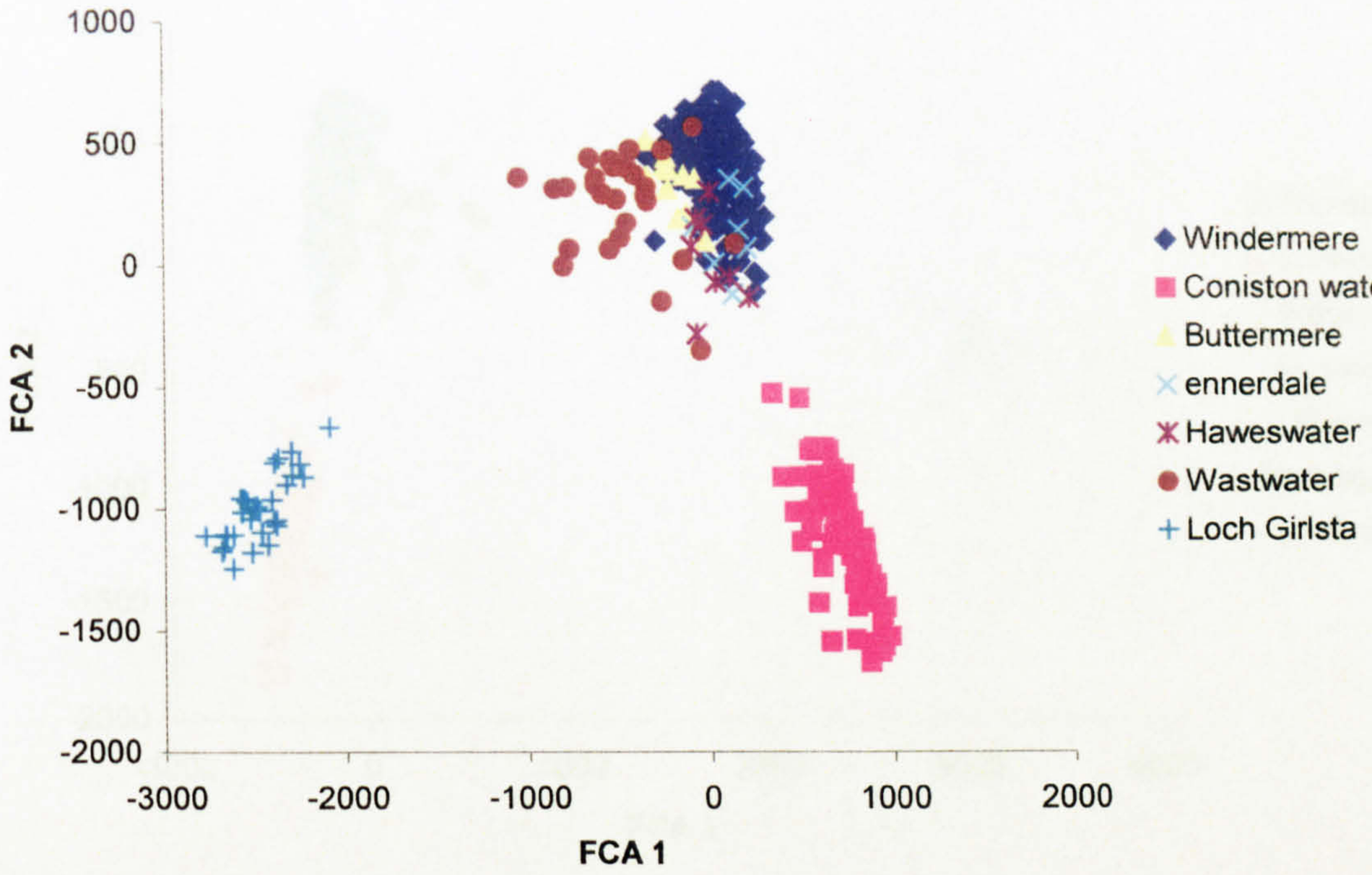
Without Omm1302



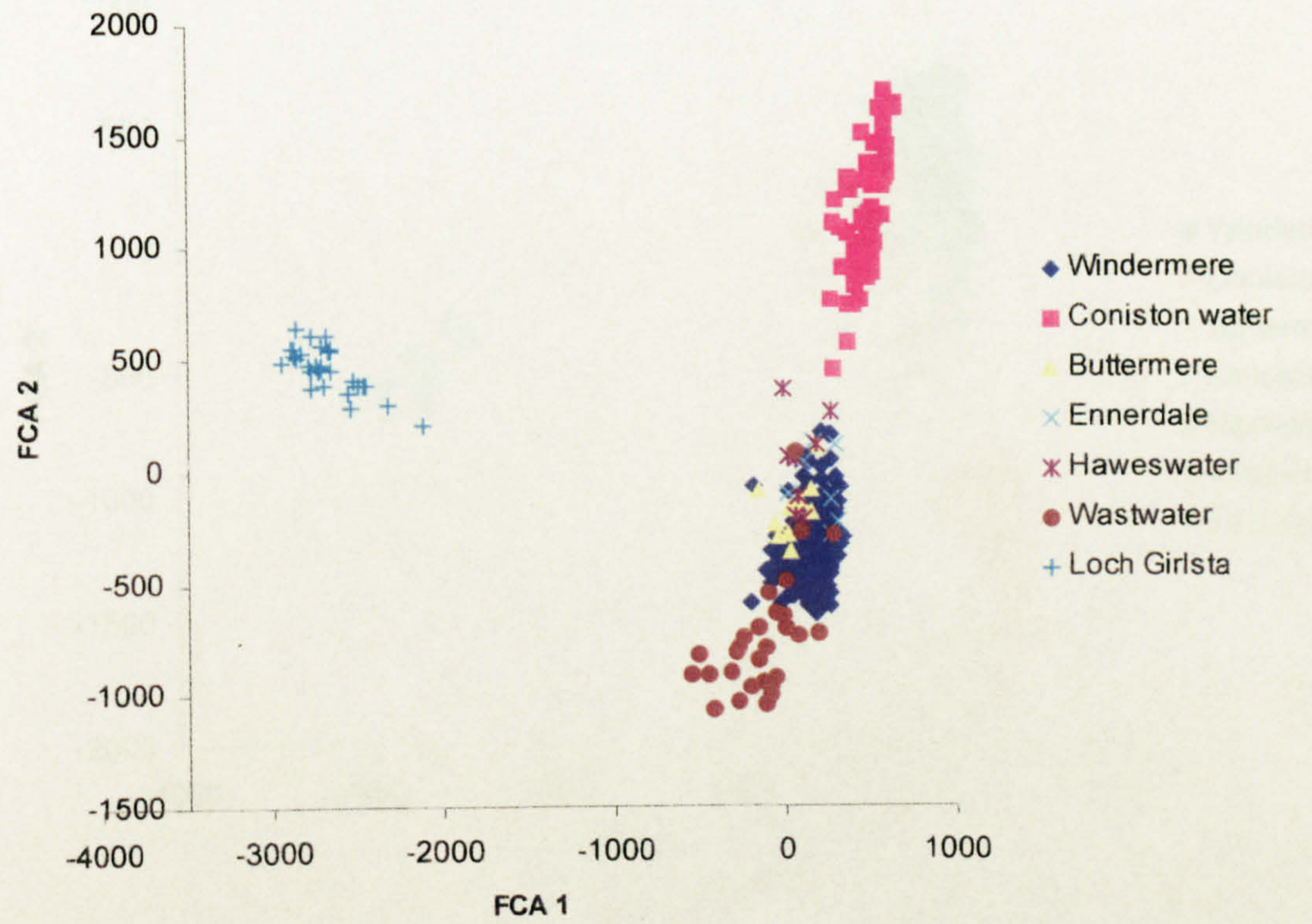
Without SalO23



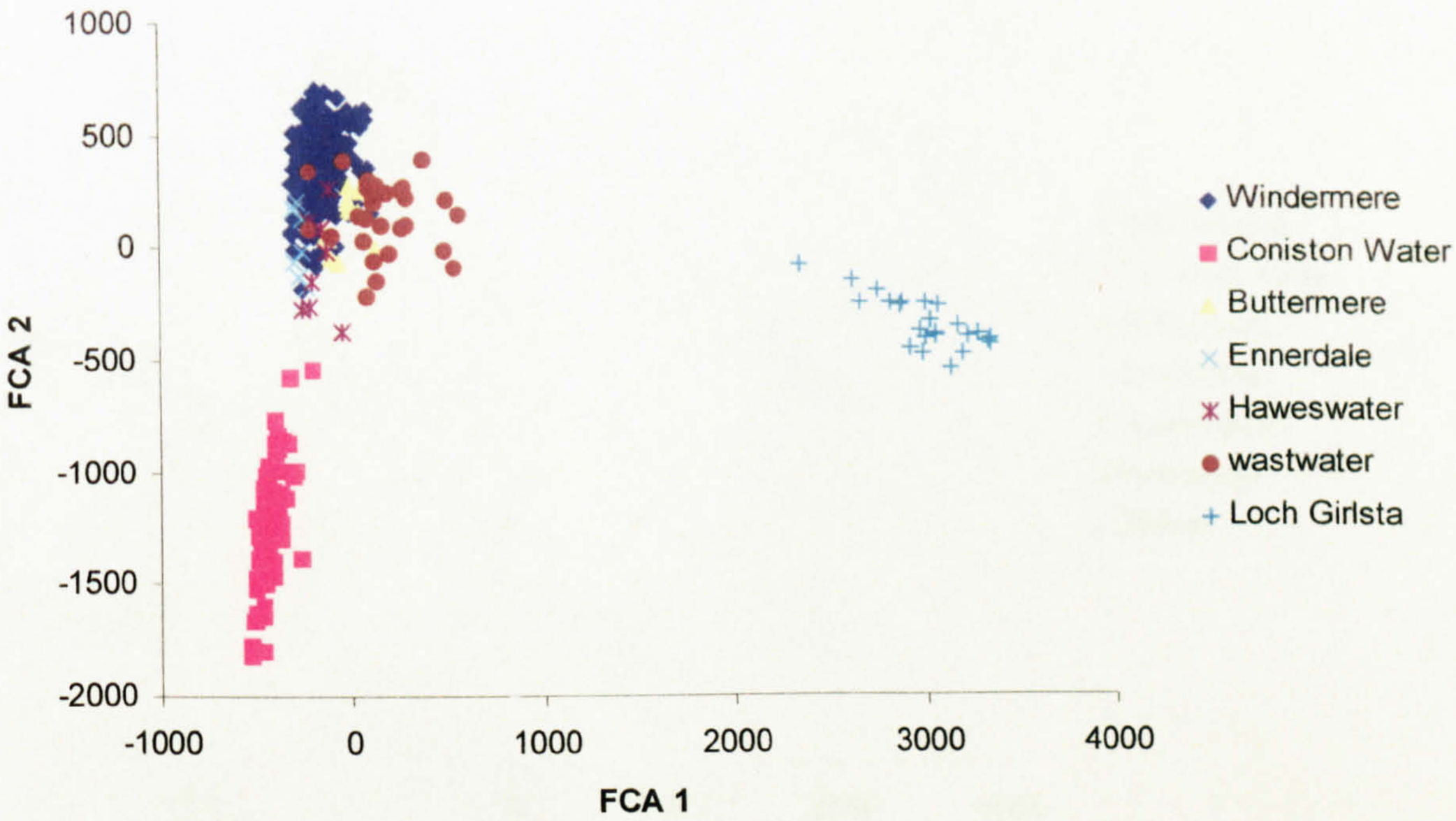
Without Omm1377



Without SalD30



Without Ssa85



Without SalJ81